HOSPITAL LIBRARY

VOLUME 83 NUMBER 4

BULLETIN of the JOHNS HOPKINS HOSPITAL

THE PUBLICATION OF THE MEDICAL SCHOOL AND HOSPITAL
SUPPORTED BY THE DE LAMAR FUND OF THE JOHNS HOPKINS UNIVERSITY

October, 1948

The Effect of Ovariectomy and Physiologic Doses of Estradiol upon Body Weight, Linear Growth, and Fat Content of the Female Albino Rat. MORTON J. NYDA, SALVADOR F. DE MAJO, AND ROGER A. LEWIS 279

Circulating Anticoagulant as a Cause of Hemorrhagic Diathesis in Man. C. LOCKARD CONLEY, HOWARD K. RATHBUN, WILLIAM I. MORSE II, AND JAMES E. ROBINSON, JR. 288

The Clinical Manifestations of the Severe Form of Diphtheria. A. MURRAY FISHER AND SIDNEY COBB 297

Antibody Formation in Alloxan Diabetes. A Comparison of the Precipitin Response to Egg Albumen of Normal Rabbits and of Rabbits with Alloxan Diabetes. TORRENCE P. B. PAYNE AND ALAN H. CRUICKSHANK 326

Comparative Study of Antihistamine Substances. I. Introduction and Dale Experiments. S. WALTER LANDAU AND LESLIE N. GAY 330

Comparative Study of Antihistamine Substances. II. Activity in Vivo Against Histamine Intoxication and Anaphylactic Shock of Guinea Pigs. S. WALTER LANDAU, HENRY J. L. MARRIOTT AND LESLIE N. GAY 343

Comparative Study of Antihistamine Substances. III. Clinical Observations. LESLIE N. GAY, S. WALTER LANDAU, PAUL E. CARLINER, N. S. DAVIDSON, FRANK F. FURSTENBERG, NATHAN B. HERMAN, WILLIAM H. NELSON, JOHN W. PARSONS AND WALTER W. WINKENWERDER 356

PUBLISHED MONTHLY

THE JOHNS HOPKINS PRESS, Baltimore-18, Maryland

Made in United States of America

COPYRIGHT, 1948, BY THE JOHNS HOPKINS PRESS

BULLETIN

OF

THE JOHNS HOPKINS HOSPITAL

MANAGING EDITOR, E. COWLES ANDRUS

ASSOCIATE MANAGING EDITOR, FREDERIK B. BANG

EDITORIAL BOARD

Charles R. Austrian
W. Halsey Barker
Kenneth C. Blanchard
Edward M. Hanrahan
John Eager Howard
Arnold R. Rich

All correspondence relating to the publication of papers should be addressed to The Editor, Bulletin of The Johns Hopkins Hospital, Baltimore-5, Md.

Books for review should be sent to The Editor at the above address.

Twenty-five reprints, without covers, of articles will be furnished to contributors free of cost. An order slip for additional reprints, with a table showing cost, is sent with proof.

Correspondence concerning business matters should be sent to The Johns Hopkins Press, Baltimore-18, Maryland, U. S. A.

This periodical is issued monthly. Two volumes a year are issued, each consisting of approximately 400 pages, and beginning in January and July, respectively.

Subscription price. In the United States and its possessions $6.00 per year. In Canada $6.25 per year; in foreign countries $6.50 per year. Prices for back volumes and back single numbers on application. Single numbers of the current volume, when available, $.75. Prices are net, postpaid.

New subscriptions and renewals are entered to begin with the first issue of the current volume. Should any issue of the current volume be out of print at the time the subscription order is received, the pro rata value of such numbers will be credited to the next volume, and the renewal invoice therefore adjusted accordingly.

Subscriptions should be renewed promptly. To avoid a break in your series, subscriptions should be renewed promptly. The publisher cannot guarantee to supply back issues on belated renewals.

Subscriptions, new or renewal, may be sent to the Publishers or ordered through your agent.

Subscriptions in the British Empire except Canada may be sent to: Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, London, W.C. 2

THE JOHNS HOPKINS PRESS
BALTIMORE-18, MARYLAND, U. S. A.

Entered as second-class matter at the Baltimore, Md., Post Office. Acceptance for mailing at special rate of postage provided for in Section 1103, act of October 3, 1917. Authorized on July 3, 1918.

THE EFFECT OF OVARIECTOMY AND PHYSIOLOGIC DOSES OF ESTRADIOL UPON BODY WEIGHT, LINEAR GROWTH AND FAT CONTENT OF THE FEMALE ALBINO RAT

MORTON J. NYDA, SALVADOR F. DE MAJO* AND ROGER A. LEWIS[1]

From the Department of Pediatrics, The Johns Hopkins University and The Harriet Lane Home, The Johns Hopkins Hospital

Received for Publication April 8, 1948

INTRODUCTION

It is well known that large doses of estrogen will retard normal weight gain and body growth of experimental animals. The purpose of this experiment is to see whether small doses of estrogen comparable to the natural supply will also cause delay in growth and weight gain of rats, and to determine whether this effect is in part due to alteration in the amount of body fat. The effect of these small doses of estrogen upon the reproductive organs and upon the blood level of calcium, phosphorus and phosphatase was also determined.

METHODS

Preliminary experiments showed that estradiol benzoate in a dosage of 30 micrograms weekly markedly retarded the weight gain of young female albino rats. Half of this dose was used in this experiment. Five micrograms of estradiol benzoate dissolved in 0.1 ml. of peanut oil were injected three times a week. The total amount of estradiol administered over the 40 day period was 85 micrograms or about 2.1 micrograms daily.

Four groups of ten female albino rats of approximately the same age and weight were observed: a control group, an ovariectomized group, and two similar groups treated with estradiol. Treatment was commenced after vaginal opening when the rats were 42–44 days old and weighed approximately 85 grams. The ovariectomized animals were allowed four days to recuperate from the operation and ether anesthesia before the treatment period was instituted.

* Eli Lilly Fellow in Medicine.

[1] Senior Fellow of the American Cancer Society recommended by the Committee on Growth of the National Research Council.

Food and water were available at all times. The diet consisted of fox chow pellets supplemented with lettuce daily and carrots once a week. The animals were weighed at five day intervals and measured before and after the treatment period. The carcasses were x-rayed at the conclusion of the experiment.

At the start of treatment vaginal smears were taken daily, later at less frequent intervals. At the conclusion of the treatment period each animal was weighed again and measured and then anesthetized with nembutal. Blood was taken from the heart and then the animal was killed and skinned. The adrenals, ovaries and uterus were

TABLE I

GROUP	WEIGHT GAIN GRAMS			LINEAR GROWTH CMS.			UTERUS WEIGHT MGS./100 GMS.			OVARY WEIGHT MGS. /100 GMS.			TOTAL FAT GMS./100 GMS.		
	Mean	Difference	S.E.*	Mean	Difference	S.E.	Mean	Difference	S.E.	Mean	Difference	S.E.	Mean	Difference	S.E.
Control	73.3			14.4			198			59			5.2		
Castrate	119.0	45.7	7.0	16.8	2.4	0.6	31	—167	28				4.9	—0.3†	0.6
Control	73.3			14.4			198			59			5.2		
Estradiol	53.6	—19.7	4.8	12.0	—2.4	0.7	293	95	40	38	—21	5	6.4	+1.2	0.4
Control	73.3			14.4			198			59			5.2		
Castrate Estradiol	69.4	—3.9†	5.2	12.3	—2.1	0.6	202	4†	28				7.3	+2.1	0.5

* S.E. or standard error of the difference of the means $= \sqrt{SE_{m1}^2 + SE_{m2}^2}$ where $SE_m = \sqrt{\frac{\epsilon\Delta^2}{n(n-1)}}$

† Not a statistically significant difference.

weighed and fixed. The hypophysis was also removed for histological study.

The skin and carcass were dissolved separately in four times their weight of 50 percent potassium hydroxide in 50 percent ethyl alcohol. After four hours of heating, the mixture was acidified with 50 percent sulfuric acid and extracted with petroleum ether. The extract was dried with sodium sulfate and filtered. The filtrate was evaporated to dryness and weighed.

RESULTS

The weight gain of the four groups of animals is shown in Table I. The average gain of the untreated animals was 73 grams in forty days

while the ovariectomized rats gained 119 grams, which was 46 grams more than the controls. The average gain of the estradiol treated animals was 54 grams, or 20 grams less than the controls. The ovariectomized rats which were treated with estradiol gained slightly less than the controls, but the difference was not statistically significant (Chart I).

The growth of the tail exceeded the growth of the body in all groups, but the difference between body growth and tail growth was quite small and in proportion to the total growth. The normal rats grew 14.4 cms. in length during the forty days. The ovariectomized rats grew 16.8 cms., an increase over normal of 2.4 cms. The estradiol treated rats grew 12.0 cms., or 2.4 cms. less than the controls. The ovariectomized and estradiol treated animals grew 12.3 cms., which is not significantly different from the other estradiol treated group but which is significantly less than the normal controls (see Table I). The differences in skeletal growth are illustrated by x-rays, Figure 1.

The uterine weight of the normal rats averaged 198 mgs. per 100 gms. body weight. Ovariectomy reduced this figure to 31 mgs., while treatment with estradiol raised it to 293 mgs. The group that was castrated and treated with estradiol had an average uterine weight of 202 mgs. per 100 gms. body weight. The ovaries in the estradiol treated rats weighed only 38 mgs. per 100 gms. body weight compared with 59 mgs. in the controls.

The total amount of fat in the skin and carcass was greater in the ovariectomized rats than in the controls, but the percent of fat in relation to the weight of the skin and carcass was lower than that of the controls. The fat in the body of the estradiol treated rats was greater in amount and percent than that of the controls although the actual difference was small, Table II. The rats that were ovariectomized and treated with estradiol showed the greatest amount and the greatest percent of fat of all groups.

The serum calcium of the pooled sera of the normal rats averaged 9.9 mgs. percent compared with 10.5 and 10.8 in the two groups treated with estradiol and 9.8 in the ovariectomized animals, see Table III. The average serum phosphorus level of the normal rats was 10.1 mgs. percent compared with 12.6 in the ovariectomized group and 11.9 and 10.2 in the estradiol treated animals. The serum alkaline phosphatase

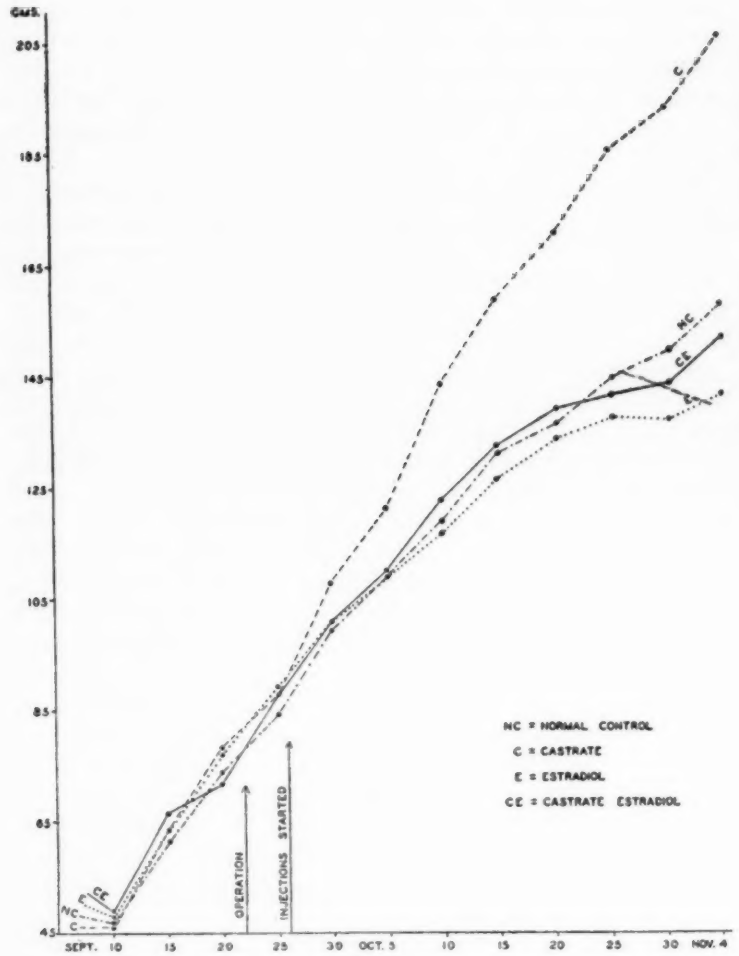


CHART I

expressed in Bodansky units per 100 cc. was 88 in the normal rats. A considerably higher value, 105, was found in the pooled blood serum of the ovariectomized rats, and a somewhat lower value was found in the two groups treated with estradiol, 69 and 71.

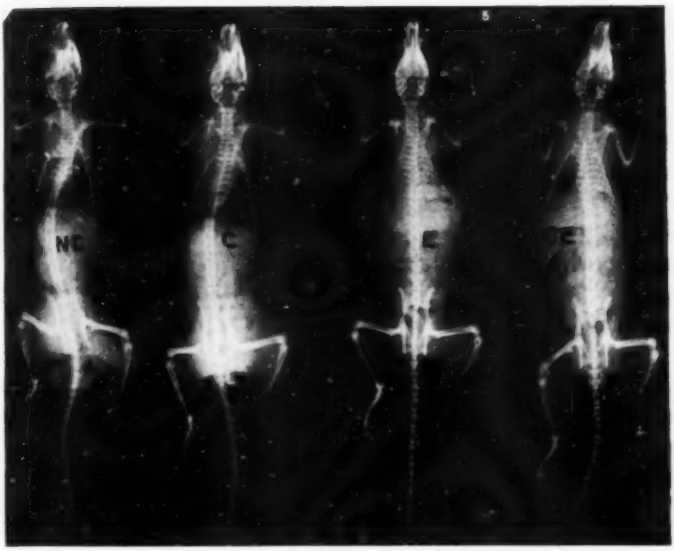


FIG. 1

NC = Normal Control
C = Castrate
E = Estradiol
CE = Castrate Estradiol

TABLE II

GROUP	SKIN			CARCASS			TOTAL ANIMAL		
	Weight	% Fat	Total Fat	Weight	% Fat	Tota Fat	Weight	% Fat	Total Fat
Normal	21.2	11.7	2.48	138	4.2	5.77	158	5.2	8.25
Castrate	29.3	10.8	3.20	178	3.9	6.96	208	4.9	10.16
Estradiol	18.3	13.6	2.48	128	5.2	6.70	143	6 4	9.18
Castrate Estradiol	18.9	14.8	2.79	137	6.4	8.82	158	7 3	11.61

TABLE III

GROUP	CALCIUM mg%	PHOSPHORUS mg%	PHOSPHATASE* B.U./100cc.
Normal	9.9	10 1	88
Castrate	9 8	12 6	105
Estradiol	10 5	11 9	69
Castrate Estradiol	10 8	10 2	71

* Alkaline phosphatase expressed in Bodansky units.

Vaginal smears taken on the control group showed a normal estrus cycle of approximately four days. The ovariectomized animals were in constant diestrus. The rats receiving estradiol were in constant estrus, although occasionally the ovariectomized rats receiving estradiol showed smears suggesting proestrus.

Histological study of the organs showed that, although there were variations in size, the structure of the adrenals was the same in all groups. The liver appeared normal in all the animals. The ovaries in the rats treated with estradiol showed the presence of many small and large cysts despite the decrease in size. The uterus was almost completely atrophied in the ovariectomized animals. In the rats treated with estradiol, both the normal and the ovariectomized groups, the uterus was normal or enlarged with prominent glands showing an increased number of vacuoles.

DISCUSSION

The fact that the ovariectomized rats gained weight more rapidly than the normal animals is in agreement with the results of other observers. Holt, Keeton and Vennesland studied ovariectomized rats and found that they had a larger food intake than normal rats of the same age (1). This might account in part for accelerated gain in weight.

Levie has shown that the administration of estrone in doses of 250–500 micrograms daily markedly suppresses gain in body weight, although in doses of 10–20 micrograms the reduction in weight gain was slight and inconstant (2). However, estrone is known to be biologically less active than estradiol. Hooker and Pfeiffer, using estradiol benzoate, found that 83 micrograms administered twice weekly, which averages 24 micrograms daily, interfered with normal weight gain (3). It is remarkable that the dose of estradiol benzoate used in this experiment, 5 micrograms three times a week, which averages 2 micrograms daily, was sufficient to retard the weight gain of normal rats and to completely counteract the increased weight gain usually shown by ovariectomized rats.

It was pointed out many years ago by Stotsenburg that in mammals the male is usually larger than the female (4) and Holt was able to produce females of larger than normal size by ovariectomy (1). The

ovariectomized rats in our experiment showed a marked increase in linear growth over that of the normals. X-ray studies showed that that there was a general increase in the size of the bones affecting the width of the skull and pelvis, as well as the length of these and the long bones.

Levie found no alteration in bone growth when doses of 5–20 micrograms of estrone were given although a definite effect was obtained with 250–500 micrograms daily (2). In the studies reported here, a dosage of 5 micrograms of estradiol benzoate three times a week, which averages 2 micrograms daily, caused a stunting of growth in normal rats of the same degree as the increase in growth shown by the ovariectomized animals. The rats that were ovariectomized and given estradiol showed the same skeletal growth as the normal animals that were treated with estradiol, showing that this dose was more than enough to counteract the effect of ovariectomy upon bone growth.

It is interesting to note that the uterus in the ovariectomized rats which were given 2 micrograms of estradiol daily was almost exactly the same size as the uterus in the control animals. The uterus in the normal rats that were treated with estradiol showed considerable enlargement beyond that of the normal. The fact that the 2 micrograms daily of estradiol approximately duplicated in ovariectomized animals the degree of uterine development which occurred in the controls does not prove that this dosage was equivalent to the normal physiological output of estrogen, because the action of estrogens may be modified by other steroids, such as progesterone.

It is frequently stated that ovariectomy causes an increase in obesity similar to that which follows castration of the male (4). However, quantitative studies showed that this increase in fat was proportional to the increase in body weight and that the percentage weight of fat was not higher in the ovariectomized animals. Holt (1) analyzed the fat content of rats twelve months after ovariectomy and found no increase compared to normal controls of the same age.

On the other hand it has been surmised that the diminished weight gain caused by the administration of estrogen might be due to a reduction in the fat deposits. However, in these experiments there was found to be a slight increase in the fat content of the estradiol treated animals, which was more striking when compared with the body weight

since these animals were the lightest group. The rats which were ovariectomized and given estradiol had the greatest amount of fat and the greatest percentage of fat. These observations are in harmony with the general observation that the female of the species has a higher percent of body fat than the male (5).

The rise in serum calcium observed in the estradiol treated rats is quite small compared with the rise observed by Pfeiffer and Gardner in birds receiving large doses of estradiol benzoate (7). The changes in serum phosphate were not as striking as the changes in alkaline phosphatase. The fact that the highest value of phosphate and phosphatase occurred in the ovariectomized group is compatible with the observation that phosphate and phosphatase are increased during the period of growth. It has been suggested that the level of serum phosphate is an indication of the production of growth hormone, and it has been shown by Albright and co-workers that estrogen diminishes the level of serum phosphate and bone growth in acromegalics (8). This is not surprising since in 1936 Zondek showed that in rats the administration of estrogen arrested growth through its effect upon the hypophysis (9). It is notable that while the phosphate level was higher in the ovariectomized rats it was not lowered by the administration of estradiol although the serum alkaline phosphatase was depressed. A depression in the serum alkaline phosphatase with estrogen treatment was noted by Day and Follis who studied the composition of rat bones after prolonged treatment with estradiol (10).

It may be concluded that, when given in physiological doses, the effect of estrogen upon weight gain is manifested chiefly through its inhibition of growth. With the decrease in growth there is a moderate increase in the proportion of body fat. This is opposite to the effect of androgen which causes an increase in growth and a decrease in the relative amount of fat (5). Furthermore, although a dose of 5 micrograms of estradiol benzoate given three times a week is just sufficient to maintain normal uterine weight, it markedly suppresses the normal skeletal growth.

SUMMARY

Female rats ovariectomized at the age of forty days showed a greater increase in both skeletal length and body weight during the

next forty days than did normal female controls of the same age. there was a slight decrease in the proportion of body fat.

The administration of estradiol benzoate in a dose of 5 micrograms Three times a week to female rats of this age caused a marked retardation of skeletal growth, a diminution in the rate of weight gain, and a considerable increase in the proportion of body fat.

Five micrograms of estradiol benzoate administered three times a week to ovariectomized rats was sufficient to maintain the uterus at the same weight as normal controls but inhibited skeletal growth. These animals showed a relative and absolute increase in body fat.

ACKNOWLEDGEMENTS

The advice and assistance of Dr. Lawson Wilkins is gratefully acknowledged. This work was aided, in part, by a grant from the Commonwealth Fund for the study of endocrine problems in childhood.

BIBLIOGRAPHY

(1) Holt, H., Keeton, R. W., and Vennesland, B.: Am. J. Physiol., **114:** 515, 1936.
(2) Gaarenstroom, J. H., and Levie, L. H.: J. Endocrinology, **1:** 420, 1939.
(3) Hooker, C. W., and Pfeiffer, C. A.: Endocrinology, **32:** 69, 1943.
(4) Stotsenburg, J. M.: Anat. Record, **3:** 233, 1909.
(5) Kochakian, C. D., Bartlett, M. N., and Trybalski, E. J.: Conference on Metabolic Aspects of Convalescence, **13:** 49, 1946.
(6) Burrows, Harold: University Press, Cambridge, 1945, page 375.
(7) Pfeiffer, C. A., and Gardner, W. U.: Endocrinology, **23:** 485, 1938.
(8) Albright, F., Reifenstein, E. C., Jr., Forbes, A. P., and Bartter, F. C.: Conference on Metabolic Aspects of Convalescence, **14:** 102, 1946.
(9) Zondek, B.: Lancet, **2:** 842, 1936.
(10) Day, H. G., and Follis, R. H.: Endocrinology, **28:** 83, 1941.

CIRCULATING ANTICOAGULANT AS A CAUSE OF HEMORRHAGIC DIATHESIS IN MAN

C. LOCKARD CONLEY[1], HOWARD K. RATHBUN[2], WILLIAM I. MORSE II[3], AND JAMES E. ROBINSON, JR.[4]

From The Clinical Microscopy Division, Department of Medicine, The Johns Hopkins University and Hospital, and The Division of Medicine, Baltimore City Hospitals, Baltimore, Maryland

Received for Publication April 14, 1948

Hemorrhagic syndromes associated with defective blood coagulation have almost invariably been attributed to deficiency of one or the other of the plasma components necessary for normal clotting. The spontaneous occurrence of an anticoagulant in the circulating blood has been rarely considered to be a cause of abnormal bleeding in human patients. Quick (11) in his extensive monograph on the hemorrhagic diseases did not mention such a condition. However, several well-studied cases have been reported in which prolongation of the clotting time and bleeding tendency have been associated with the presence of a clotting inhibitor in the circulating blood (2, 3, 4, 6, 8, 12). We have had the opportunity to study three patients in whose blood an anticoagulant has been demonstrated.

ABSTRACTS OF CASES[5]

1. J. H., (B. C. H. #114201), a 67 year old senile colored male was admitted to the Baltimore City Hospitals in October, 1947, because of hematuria of several weeks duration. There was no family history of hemorrhagic tendency and no previous history of abnormal bleeding. Genito-urinary study revealed no demonstrable cause of the hematuria. Because the patient had moderate generalized glandular enlargement, a lymph node biopsy was performed. This procedure was followed by profuse bleeding from the wound for several days. Sections of the node showed only hyperplasia. Defective blood coagulation was observed before any transfusions were given. The venous clotting time was 68 minutes. Recalcified plasma clotting time was prolonged to 12 minutes (normal about 2

[1] Instructor in Medicine, The Johns Hopkins University School of Medicine.
[2] Assistant Chief, Division of Medicine, Baltimore City Hospitals.
[3] Assistant in Pathology, The Johns Hopkins University School of Medicine.
[4] Assistant Resident Physician, Baltimore City Hospitals.
[5] Cases 1 and 2 will be described in detail in subsequent reports.

minutes). The prothrombin time was normal. Plasma fibrinogen concentration was 0.21 grams per 100 cc. Platelet count, bleeding time and capillary fragility were within the limits of normal.

2. A.V., (J. H. H. #445357), a 39 year old white farmer was admitted to The Johns Hopkins Hospital in December, 1947, because of vague abdominal pain, malaise, and weight loss of three months' duration. There was no family history of hemorrhagic diathesis and no past history of abnormal bleeding. He had had a herniorrhaphy, appendectomy, and hemorrhoidectomy prior to 1944 without unusual bleeding. He had never received a transfusion. In 1942 he was found to have a positive serologic test for syphilis for which he was treated. In 1944, while in the army, he was hospitalized for trench foot and was reported to have a cardiac murmur and "chronic glomerulo-nephritis". In November 1947 he had two episodes of hemoptysis without other definite pulmonary symptoms. Physical examination showed apical systolic and presystolic murmurs and slightly enlarged spleen The urine contained many red cells and variable amounts of albumin. A sero'logic test for syphilis was positive. X-ray of the chest showed no abnormality of the heart or lungs. While in the hospital he had two more episodes of hemoptysis and occasionally complained of abdominal pain. A definite diagnosis was not established. Venous clotting time was 60 minutes, recalcified plasma clotting time 7½ minutes, prothrombin time 30 seconds (normal 16 to 20 seconds). The plasma fibrinogen concentration was 0.53 grams per 100 cc. Platelet count, bleeding time, and capillary fragility were normal.

3. F. Y., (J. H. H. #104833), a 38 year old hemophiliac was admitted to The Johns Hopkins Hospital in February 1948 for tooth extraction. Several maternal uncles and two brothers had hemophilia. The patient had had 10 previous admissions for various hemorrhagic manifestations. He had received numerous transfusions, and on his last admission in August, 1947 had been given both blood and anti-hemophilic globulin. Venous clotting time was 5 hours, recalcified plasma clotting time 17 minutes, prothrombin time normal. Platelet count, bleeding time, and capillary fragility were normal.

EXPERIMENTAL STUDIES

Demonstration of the presence of anticoagulant in plasma

Blood was obtained from each of the three patients using silicone-treated needles, syringes and tubes. After centrifugation in an angle centrifuge at 5000 to 12,000 RPM for about 20 minutes, the supernatant plasma was removed. This plasma was essentially platelet-free and failed to clot even when stored for days in glass tubes. In this manner, permanently fluid native plasma was obtained without the necessity of adding anticoagulant to the blood. The presence of spon-

taneously occurring anticoagulant was demonstrated by adding small amounts of this plasma to freshly drawn normal human blood and observing the effect on the clotting time. The results of two assays on each of the three patients are shown in Table 1. For comparison there is included in the table an identical study on the plasma of a hemophiliac (A. B.), who had no demonstrable anticoagulant in his blood.

It is apparent that each of the three patients had a potent clotting inhibitor in his plasma. The anticoagulants in the plasmas of J. H.

TABLE 1

Effect on the clotting time of normal human blood of admixture with plasma from our 3 patients

Patient A. B. was an ordinary hemophiliac without circulating anticoagulant, included for comparison. All clotting times were done by a 3 tube method, the recorded time being that of the third tube. Addition of 0.5 cc. or less of 0.85% sodium chloride solution to 1 cc. normal blood had no effect on the clotting time; therefore, data for the saline controls are not included in the table.

SOURCE OF PLATELET-FREE PLASMA	DATE	CLOTTING TIME IN MINUTES OF 1 CC PORTIONS OF NORMAL BLOOD AT 37°C AFTER ADDITION OF PLATELET-FREE PLASMA FROM PATIENT VOLUME OF PLASMA ADDED				
		0	0.01 cc.	0.1 cc.	0.2 cc.	0.5 cc.
J. H.	12-10-47	14		39	67	
	3- 1-48	7	32	40	43	
A. V.	12-29-47	13		39	75	75
	1-27-48	13	20		57	
F. Y.	2-17-48	14				68
	2-19-48	18		25	31	54
A. B.	1-13-48	8		9	9	8

and of A. V. were of such a titer that one part of the patient's plasma in 100 parts of normal blood prolonged very appreciably the clotting time of the latter. In the case of F. Y., one part of the patient's plasma in ten parts of normal blood was required to produce an unequivocal delay in clotting.

Nature of the anticoagulants

Several studies were carried out in an attempt to identify the clotting inhibitors in the blood of these patients. In an effort to demonstrate

a possible relationship of the anticoagulants to heparin, the effect of toluidine blue and of protamine on the clotting time was observed. Serial concentrations of toluidine blue and of protamine, which were very effective in shortening the clotting time of heparinized normal blood, were used. However, there was no significant effect of these agents on the clotting time of blood from these patients.

Assays of the plasma for proteolytic enzyme and for proteolytic enzyme inhibitor were made by Dr. Oscar D. Ratnoff and Dr. David Grob. Essentially normal values of both were obtained in each instance.

In the case of F. Y., the hemophiliac, precipitin tests were set up with the patient's serum against normal plasma and against a solution of Fraction I of Cohn (containing antihemophilic globulin). No precipitins could be demonstrated.

Platelet-free plasma from each of the patients was dialyzed against 0.85% sodium chloride solution without loss of anticoagulant activity. In each case the anticoagulant withstood heat at 65°C. for 5 minutes.

Antithrombic activity of the anticoagulants

Tests were performed to determine the phase of blood coagulation in which the anticoagulants exerted their effects. The prothombin times of patients J. H. and F. Y. were within the normal range and it seemed unlikely that their anticoagulants could be antithrombic. However, the prothrombin time of A. V. was moderately prolonged.

Oxalated plasma from each patient was mixed with a solution of thrombin. The clotting times obtained were compared with the clotting times of a series of normal plasmas similarly treated. Table 2 shows the results of this test.

The data reveal that none of the plasmas showed an increase in antithrombic activity. Nevertheless, it seemed probable that the prolonged prothrombin time of patient A. V. might be accounted for by the presence in his blood of an anticoagulant rather than by a deficiency of prothrombin. Evidence for this was provided by another type of examination.

Oxalated plasma from each of the patients was treated with barium sulfate to remove all of the prothrombin. The prothrombin-free plasmas thus obtained were used as diluents for a normal oxalated

plasma, one part of the normal plasma being added to four parts of prothrombin-free plasma in each instance. The actual prothrombin concentration in each of these mixtures was 20% of the normal, since all of the prothrombin present was supplied by the portion of normal plasma. As a control, a portion of the normal plasma used in the test

TABLE 2

Clotting time at 37°C. of 0.1 cc. oxalated plasma on addition of 0.1 cc. bovine thrombin solution

Each figure represents the mean of several determinations. The tests on the 3 patients were done on different days with different thrombin solutions and therefore cannot be compared with each other but only with the accompanying control value. Several normal control plasmas were used in each instance and the results averaged.

	J. H.	NORMAL CONTROLS	A. V.	NORMAL CONTROLS	F. Y.	NORMAL CONTROLS
Thrombin time (seconds)	27	30	34	38	34	36

TABLE 3

The prothrombin time of a normal plasma diluted to 20% with normal prothrombin-free plasma compared with an equal concentration of the same normal plasma diluted with prothrombin-free plasma from the patient

The tests on the 3 patients were done on different days with different normal plasmas and cannot be compared with each other but only with the accompanying control.

	SOURCE OF PROTHROMBIN-FREE PLASMA					
	J. H.	Normal Control	A. V.	Normal Control.	F. Y.	Normal Control
Prothrombin time of normal plasma diluted to 20% with prothrombin-free plasma (seconds)	41	40	50	33	46	48

was also treated with barium sulfate and was similarly employed as a diluent for the untreated normal plasma. The results of the prothrombin time determinations on these plasma mixtures are shown in Table 3.

When the prothrombin-free plasma of patient J. H. or of F. Y. was used as a diluent for a normal plasma, essentially the same prothrombin

time was obtained as when a normal prothrombin-free plasma was used as a diluent. However, prothrombin-free plasma from patient A. V. definitely prolonged the prothrombin time of the normal plasma. It seems evident that the prolonged prothrombin time of patient A. V. was the result of the presence in his blood of a clotting inhibitor.

Antithromboplastic activity of the anticoagulants

The antithromboplastic activity of plasma was estimated by determination of the clotting time of platelet-free plasma on addition of a suspension of acetone-extracted rabbit brain thromboplastin. The

TABLE 4

Clotting time in seconds (37°C.) of platelet-free plasma (silicone technic, no anticoagulant added) on addition of an emulsion of acetone-extracted rabbit brain thromboplastin

Different thromboplastin preparations were used in the 2 tests which are therefore not comparable. In test II the plasma of an ordinary hemophiliac (Hem) was used for comparison instead of normal plasma.

TEST	SOURCE OF PLATELET-FREE PLASMA	THROMBOPLASTIN DILUTIONS					
		Undilute	1:10	1:50	1:100	1:200	1:400
I	Normal	15	19	56	108	240	320
	A. V.	25	71	>600	—	—	—
	F. Y.	15	21	66	170	307	419
II	Hem.	15	27	58	83	160	335
	J. H.	15	23	45	56	79	138
	A. V.	23	77	196	250	525	—

plasmas used in the test were obtained by the silicone technic in the same manner as for the anticoagulant assay previously described. Although the platelet-free plasma from each of the patients could be kept indefinitely in glass tubes without evidence of clotting, coagulation occurred promptly on addition of thromboplastin. Serial dilutions of the thromboplastic preparation were used in the test. Table 4 shows the results of two of these assays.

The response to thromboplastin of the platelet-free plasmas of J. H. and F. Y. appeared to be essentially normal. However, the clotting times of the plasma of A. V. were considerably prolonged. The plasma of A. V. has already been shown to contain a clotting inhibitor which

delays the conversion of prothrombin to thrombin. Whether this inhibitor is actually antithromboplastic, or whether in some other way it inhibits activation of prothrombin, could not be established. However, the results indicate that with decreasing concentrations of thromboplastin the effect of the clotting inhibitor was much more evident. When oxalated plasma from patient A. V. was incubated with thromboplastin before recalcifying, there was no evidence of inactivation of thromboplastin.

DISCUSSION

A number of case reports have appeared in the literature in which atypical hemorrhagic diatheses have been found to be associated with prolongation of the coagulation time. In most of these cases the cause of the delay in clotting was not determined. For example, Madison and Quick (7) in a recent paper describe a case and mention several others in which a hemophilia-like disease was observed in the female. In these cases no anticoagulant assay was reported. It seems not improbable that in at least some of these patients a circulating anticoagulant may have been the cause of the coagulation defect.

In 1940, Lozner et al. (6) reported the case of a 61 year old mulatto with a hemorrhagic disorder associated with the presence of a circulating anticoagulant. The patient was found to have a positive serologic test for syphilis and glandular tuberculosis. The source of the anticoagulant was not determined. The anticoagulant was relatively thermostabile and non-dialyzable. Its effect was not altered by protamine and therefore it did not appear to be heparin. The prothrombin time was reported to be normal, so that it seems likely that the anticoagulant must have interfered with the first stage of coagulation.

A circulating anticoagulant developing in the plasma of hemophiliacs following repeated transfusions has been described by Lawrence and his associates (3, 5) and by Munro (8, 9, 10). Three such cases have now been reported. Study of these cases showed that the anticoagulant was associated with the gamma globulins. Craddock and Lawrence (3) were able to demonstrate in the sera of their patients precipitins against normal plasma and against Cohn's Fraction I containing the antihemophilic globulin. They believe that the anticoagulant in the hemophiliac is an antibody against the antihemophilic globulin.

Chargaff and West (2) reported the case of a woman with a hemophilia-like disorder in whom the prolonged clotting time was caused by a circulating anticoagulant. The anticoagulant was not inhibited by protamine. It was not antithrombic, but its effect was overcome by tissue extract thromboplastic protein. No cause for its appearance in the blood was found.

A hemorrhagic disorder in a 39 year old female was described by Fantl and Nance (4). The patient was shown to have a circulating anticoagulant which appeared to be antithromboplastic for human brain thromboplastin but not for rabbit brain. There was no antithrombic activity. The bleeding tendency in this patient appeared several months after a normal pregnancy.

Castex (1) mentioned briefly 5 cases of "pseudohemophilia" with prolonged coagulation time in which the clotting time was shortened in vivo and in vitro by protamine. He attributed the disorder to the presence of heparin in the blood. The clinical situations were not described.

The anticoagulants which we have described appear to be different in each of the three cases. If the thesis of Craddock and Lawrence is accepted, the clot inhibitor in the hemophiliac is of antibody nature, developing as a result of therapeutic administration of a protein present in normal blood but foreign to the hemophiliac. We were unable to demonstrate precipitins against antihemophilic globulin in our hemophilic patient, but a relationship between previous transfusions and the production of the anticoagulant seems not unlikely. Patient J. H., whose anticoagulant resembled that of the hemophiliac in its mode of action, had not been transfused before his coagulation disorder occurred. His anticoagulant must have been produced by some other mechanism. The clotting inhibitor of A. V. was very different from the others in its mode of action. The anticoagulant in each patient was relatively heat stable, non-dialyzable and probably protein in nature.

The anticoagulants of patients J. H. and F. Y. did not appear to be antithrombic nor antithromboplastic. Their action, therefore, must precede the liberation of active thromboplastin in the blood. It seems probable that they exert their effect by preventing the conversion of a thromboplastin precursor to its active state. On the other hand, the clotting inhibitor in the plasma of A. V. definitely delayed the con-

version of prothrombin to thrombin even in the presence of an excess of active thromboplastin.

The discovery of cases such as we have reported emphasizes the importance of carrying out anticoagulant assays on the blood of all patients with prolonged coagulation time. It seems probable that these cases are not as rare as a survey of the literature would lead one to believe. No doubt many of the reported cases of hemophilia-like disorders could have been shown to be of this type. These cases present a serious problem with regard to therapy. The anticoagulants are so potent that their effects obviously could not be overcome even by massive transfusions. We know of nothing which will serve as an antidote to their actions.

SUMMARY AND CONCLUSIONS

1. Three cases are reported in which a hemorrhagic diathesis with prolonged clotting time has been found to be associated with the presence of an anticoagulant in the blood.

2. Studies on the nature and mode of action of these anticoagulants are described.

3. The importance of performing anticoagulant assays on the blood of patients with prolonged clotting times is emphasized.

REFERENCES

1. Castex, M. R.: Bull. Acad. Med. Paris **130:** 596, 1946.
2. Chargaff, E. and West, R.: J. Biol. Chem. **166:** 189, 1946.
3. Craddock, C. G., Jr., and Lawrence, J. S.: Blood **2:** 505, 1947.
4. Fantl, P. and Nance, M. H.: Med. J. Australia **2:** 125, 1946.
5. Lawrence, J. S. and Johnson, J. B.: Tr. Am. Climat. & Clin. Assn. 1941.
6. Lozner, E. L., Jolliffe, L. S. and Taylor, F. H. L.: Am. J. Med. Sc. **199:** 318, 1940.
7. Madison, F. W. and Quick, A. J.: Am. J. Med. Sc. **209:** 443, 1945.
8. Munro, F. L. and Jones, H. W.: Am. J. Med. Sc. **206:** 710, 1943.
9. Munro, F. L.: J. Clin. Invest. **25:** 422, 1946.
10. Munro, F. L. and Munro, M. P.: J. Clin. Invest. **25:** 814, 1946.
11. Quick, A. J.: The Hemorrhagic Diseases and The Physiology of Hemostasis, Charles C. Thomas, 1942.
12. Quick, A. J.: Fed. Proc. **7:** 180, 1948.

THE CLINICAL MANIFESTATIONS OF THE SEVERE FORM OF DIPHTHERIA

A. MURRAY FISHER AND SIDNEY COBB

From the Biological Division, Department of Medicine, The Johns Hopkins University and Hospital, and Sydenham Hospital and Department of Preventive Medicine, Johns Hopkins University

Received for Publication April 20, 1948

I. INTRODUCTORY REMARKS

It is well known that diphtheria is caused by the growth of virulent C. diphtheriae organisms in various tissues of a susceptible host, resulting in the elaboration and absorption of a soluble exotoxin capable of producing serious damage to some of the body's most vital cells. The primary infection in the majority of cases occurs in the respiratory tract, though lesions in the skin are not uncommon and other sites are occasionally involved. This primary infection is an intense inflammatory reaction producing edema of the surrounding tissues and usually a tough, membranous type of superficial exudate. This *bacterial* infection tends to be a localized one, and though the whole respiratory tract may often be involved from the nares to the bronchioles, invasion of the deeper tissues by the organisms with suppuration or spread into the lymphatics or blood stream almost never occurs. The *toxin*, on the other hand, is rapidly absorbed and is responsible for the systemic symptoms characteristic of the disease. Certain types of cells—particularly those of the myocardium, the nervous system and the adrenals—seem to be more susceptible to its action than most of the other tissues of the host. The therapeutic approach to the problem, therefore, is concerned with relief of obstructive symptoms caused by this peculiar type of inflammation in the respiratory tract, and always calls for neutralization of the circulating toxin as quickly as possible, before it is fixed by the susceptible cells.

Because of the extensive programs that have been instituted throughout the whole country, and especially in the large cities, for control of diphtheria by immunization with toxoid, there is a dangerous tendency for the medical profession as a whole to feel safe regarding this in-

fection. However, diphtheria still presents a problem of great importance to the general medical profession, and several reasons may be given here in support of this statement.

1. There has been an increase in prevalence of diphtheria throughout the United States in the past few years (1). This certainly has been true in Baltimore, where in 1944 there were 227 cases with 13 deaths; in 1945, 352 cases with 19 deaths, and in 1946, 419 cases with 20 deaths. This is in contrast to the situation in 1943 when the incidence was 109 cases with 3 deaths and in 1942 when 74 cases were reported, with no deaths. In 1947 the incidence dropped again, there being 143 cases with 4 deaths (2). In considering these outbreaks and the common occurrence of the severe and even malignant form of diphtheria, one wonders at once whether or not a new type of corynebacterium may be responsible. However, no evidence has been obtained to indicate that this can be related either to strain difference or to an increase in virulence of the organisms. There is a definite possibility, however, that such outbreaks may be due, at least to some extent, to the large influx of war workers from rural areas; such groups being, presumably, more susceptible to the infection, due either to lack of immunization or lack of previous contact with the organisms.

2. A second point that deserves emphasis is that diphtheria is too often considered a problem primarily for pediatricians. However, one purpose of the present study is to show that such an attitude is neither a logical nor a safe one, for there have been many instances of the infection in young adults and even in patients in the older age groups. There actually seems to be a tendency to a rising age incidence; this is fairly definite in the Baltimore statistics and there seems to be a similar trend in other parts of the country. These figures likewise must have been influenced to some extent by the crowding of non-immune war workers into the large cities. This point of age incidence is further emphasized when we consider the common occurrence of diphtheria outbreaks in the service personnel during the years of the War; such outbreaks having been encountered in all parts of the world.

3. It has likewise been noted in the recent epidemics in Baltimore that many of the patients gave a definite history of previous immunization (3). This same phenomenon was reported in 1946 from a Canadian Province (4) and also in a "highly immunized community" in

Great Britain in 1947 (5). Consequently, one cannot rely on a history of toxoid administration to rule out the diagnosis of diphtheria; nor can one always rely on a history of a previous attack of diphtheria to rule out the possibility as there are occasional instances in which two definite attacks have occurred in the same individual.

4. There is another fact that has been thoroughly established but often lost sight of, namely that most cases of diphtheria can be cured if recognized early and *treated immediately* with adequate amounts of antitoxin. The great importance of the time factor does not seem to be adequately appreciated. The diphtheria toxin is comparable in many ways to that of tetanus, and also to many viruses. If it is not inactivated by antibodies it is taken up by susceptible cells and evidently fixed by them in such a way as to be protected from the antibodies. Its damaging effect on these cells may not be apparent at once but may appear a few days or weeks later as some of the serious delayed manifestations of the infection. A good example of the way in which diphtheria toxin may be fixed rapidly by cells is shown in the virulence test in which the reaction is carried out in the skin of the rabbit. In this test a small amount of the culture filtrate is injected into the shaved abdominal skin of the rabbit, and 5 hours later a large dose of antitoxin is given intravenously. In a positive test, in spite of the presence of a great excess of antibodies in the circulating blood, a wide area of skin shows signs of an acute inflammation, which reaches its height long after the antitoxin has been injected. In other words, though the spread of the toxin may be limited in this manner and its systemic effects prevented, the local *cellular* reaction continues, apparently uninfluenced by the antioxin. Consequently, if we are justified in comparing this reaction to the natural infection, we have an additional argument for the possible life-saving effect of diagnosing and treating diphtheria in its earliest stages, before damage to vital cells has become too great or irreversible.

II. THE CLINICAL PICTURE OF DIPHTHERIA NOTED IN A SERIES OF 30 CASES RECENTLY OBSERVED

The object of the present study was to point out the clinical and pathologic features of the more severe forms of diphtheria by an analysis of a group of 30 cases which have recently come under observation.

Five of these patients were treated at The Johns Hopkins Hospital and 25 at The Sydenham Hospital for Infectious Diseases. They were all seen in the past few years, and except for 3 of those at The Johns Hopkins Hospital they were seen in 1945–1947. The selection of cases was based on their ability to demonstrate the salient features of the severe form of the acute illness which we wished to point out. There were no children in this group under the age of 3 years and many were older children or adults. In our selection we tended to pick out a group of patients who were older than the average of those admitted to The Sydenham Hospital. Consequently, though there has been an increase in the age level of patients with diphtheria in Baltimore in the past few years, our figures in this small series are not meant to emphasize that point. Some of the cases were problems in diagnosis, most of them showed the dangerous complications or manifestations that may occur early or late, and a few served to illustrate the "malignant" type of infection. The chief points of interest are illustrated in Table I.

From a consideration of the data shown in the table, and from more complete analysis of the patients' records, the clinical picture of diphtheria as demonstrated by this group of cases may be analyzed as follows:

(A) *History* and *Symptoms:* Aside from a story of exposure to a known or suspected case of diphtheria, there is little of real diagnostic importance in the patients' symptoms that will differentiate this from other acute infections of the upper respiratory tract. The initial symptoms are usually sore throat and rather marked prostration, while the temperature is only moderately elevated. Vomiting is a common occurrence, particularly in children, but probably not more so than in other acute infections. Following this there are frequently symptoms of partial obstruction to the nares, pharynx or larynx depending on the location of the infection. A sanguineous discharge from the nose is very suggestive of diphtheria, though its incidence is not high. The diagnosis is seldom suspected during the first 2 or 3 days, and the patients are usually given symptomatic treatment or one of the sulfonamides. As was mentioned previously, a good proportion of the cases in these recent outbreaks has been in patients giving a history of immunization with toxoid—12 out of 30 being in that category in our

series. This problem deserves further analysis and study in order to determine whether or not enough doses of toxoid had been given, whether the standard toxoid is maximally antigenic, etc.

Another point which serves to emphasize the fact that the history (and even the preliminary examination of the patient) does not lead to an early diagnosis is shown by the lapse of time before antitoxin is given. In these 30 patients the duration of the symptoms prior to diagnosis and treatment varied from 2 to 17 days, with an average of 4.7 days. The chief reason, however, for this failure to make an early diagnosis in diphtheria is not so much the lack of specificity of the symptoms and signs as it is the failure of the physician to consider this as a prominent possibility in all patients with acute infections of the upper respiratory tract.

(B) *Physical Findings:* The patient with diphtheria is apt to appear more ill and prostrated than one would expect from the temperature elevation and there is often tachycardia which is out of proportion to the fever, in comparison with other acute respiratory infections. A bloody nasal discharge is a very important finding from a diagnostic standpoint—as mentioned earlier. The *local lesion*—whether it be in the nose, throat or larynx—is usally a tough, membranous type of exudate attached firmly to the tissues and tending to be confluent rather than spotty or punctate. The borders of the "membrane" are usually fairly sharply demarcated and the surrounding tissues—particularly those in the pharynx—show varying degrees of edema and redness. This edema at times tends to be so marked as to make it nearly impossible for one to get a good view of the posterior pharyngeal wall and the exudate itself may be entirely hidden. It is in this type of case that the diagnosis of diphtheria is frequently overlooked and precious time may be lost in waiting for the culture reports. The most typical local lesions are those in which there is a membrane in the nares or the larynx, or on the posterior pharyngeal wall and extending up over the soft palate and uvula. In such cases a positive diagnosis can be made at once and antitoxin given immediately.

Cervical adenitis of greater or lesser degree is present in nearly all patients, and in the tonsillar or pharyngeal types it often becomes very marked. Frequently in the patients who are severely ill the adenitis is accompanied by a marked edema that is brawny in character, tender

TABLE I

Summary of Clinical, Laboratory, and Other Data in 30 Cases of Diphtheria

NAME AND NUMBER	AGE	PREVIOUS IMMUNIZATION (AGE)	VIRULENCE TESTS[1]	OTHER BACTERIA (1)	DURATION (IN DAYS) PRIOR TO ANTITOXIN	N.P.N. (HIGHEST DETERMINATION)	OUTCOME (2)	CHIEF CLINICAL AND PATHOLOGICAL FINDINGS		
								Clinical Features		Significant Autopsy Findings (exclusive of local lesions)
								Cardiac abnormalities (3)	Other manifestations or complications	
1. H. A., 278665[1]	14	?	+	β-strep.	4	88	R	+	Laryngeal obstruction. Bullneck. Acute Nephritis (4)	
2. C. O., 287990	51	?	−	−	4		R	+	Laryngeal obstruction. Bullneck.	
3. A. F., 34685	10	+ (6 mos.)	−	β-strep.	2		R	0	"Peritonsillar abscess" (5). Bullneck.	
4. J. R., 343667	17	0	+		4	150	D (13 days)	+	Laryngeal obstruction. Diabetes mellitus (severe) Acute Nephritis (4).	
5. I. G., 355002	34	?	+	Staph. aureus	5	38	R	++	Palatal Paralysis. Peripheral Neuritis.	
6. J. G., 380105	35	?	+	−	3	57	D (2 days)	++++	Circulatory Collapse. Laryngeal obstruction. Bullneck.	Adrenal Cortical damage (6). Petechial hemorrhages in myocardium.

7. K. G., 33999	6	+ (6 mos.)	+	α-strep.	9	70	D (34 days)	++	Laryngeal obstruction. Atelectasis. Diaphragmatic paralysis.	Myocarditis (lesions showing healing & necrosis). Lobular pneumonia.
8. R. H., 33975	27	0	+	−	2		D (4 hrs.)	0	Laryngeal obstruction. Bullneck.	Laryngeal obstruction. Atelectasis (Slight)
9. A. F., 33874	59	0	+	β-strep. α-strep.	4		D (1 day)	++	Arteriosclerotic Heart Disease.	
10. C. K., 33773	11	+ (9 yrs.)	+	α-strep.	3		R	++	Palatal and ocular Paralysis.	
11. F. K., 34174	4	0	−	α-strep.	6		D (6 days)	+++	Circulatory Collapse. Laryngeal obstruction.	
12. J. G., 34036	7	+ (5 yrs.)	+	−	4		D (6 days)	++++	Circulatory Collapse. Bullneck. Anemia.	
13. J. K., 33958	28	0	+	β-strep. staph.	17	165	D (65 days)	++++	Laryngeal obstruction. Polyneuritis. "Peritonsillar Abscess" (5).	Myocarditis (extensive and healed) Lobular Pneumonia.
14. R. L., 33658	18	?	+	α-strep. β-strep. Pneumo.	2		D (10 days)	++++	Circulatory Collapse. Tracheobronchial obstruction. Old Poliomyelitis.	Myocarditis. Lobular Pneumonia. Adrenal Cortical damage (slight)
15. M. R., 33840	12	+ (6 yrs.)	− +	α-strep. β-strep.	5		D (9 days)	+++	Circulatory Collapse. Palatal paralysis. Bullneck.	Myocarditis. Adrenal cortical damage.

TABLE I—*Continued*

NAME AND NUMBER	AGE	PREVIOUS IMMUNIZATION (AGE)	VIRULENCE TESTS[2]	OTHER BACTERIA (1)	DURATION (IN DAYS) PRIOR TO ANTITOXIN	N.P.N. (HIGHEST DETERMINATION)	OUTCOME (2)	CHIEF CLINICAL AND PATHOLOGICAL FINDINGS		
								Clinical Features		Significant Autopsy Findings (exclusive of local lesions)
								Cardiac abnormalities (3)	Other manifestations or complications	
16. J. K., 34464	11	0	+	α-strep. β-strep. (few)	2		D (5 days)	++++	Circulatory Collapse. Bullneck.	
17. W. B., 34352	6	0	−	α-strep. β-strep.	3		D (1 day)	+	Bullneck.	
18. H. S., 33678	4	+ (3 yrs.)	+ (trachea) − (nose)	−	5	64	D (1 day)	++++	Laryngeal obstruction. Bullneck.	
19. R. B., 33691	3	+ (1 yr.)	+	α-strep. β-strep.	5	103	D (2 days)	+	Tracheo-bronchial obstruction. Bullneck.	Purulent Bronchitis. Lobular Pneumonia. Atelectasis.
20. R. R., 34746	11	+ (5 yrs.)	+	β-strep.	4		R	+++	Bullneck	
21. D. K., 33776	6	+ (5 yrs.)	+	α-strep.	5	104	D (4 days)	+++ (With failure)	Circulatory Collapse. Bronchopneumonia. Laryngeal & Tracheal obstruction. Bullneck.	Myocarditis. Adrenal cortical damage. Lobular Pneumonia.
22. P. W., 34492	9	0	+	β-strep.	5	206	R	+	Bullneck. Nephritis (4).	

23. T. L., 34763	7	+ (2 mos. ago)	−	Pneumo. VI. β-strep. α-strep.	4	154	D (12 days)	++++	Circulatory Collapse. Bullneck.	
24. K. W., 35040	17	0	+	β-strep.	3	60	D (11 days)	+++ (With failure)	Circulatory Collapse. Pharyngeal Paralysis. Bullneck	Myocarditis. Adrenal cortical damage. Atrophy of one adrenal
25. G. K., 35154	6	?	+	α-strep.	2	86	D (3 days)	++++	Circulatory Collapse.	Myocarditis. Adrenal cortical damage. Lobular Pneumonia.
26. B. S., 33554	17	+ (6 yrs.)	+	α-strep. β-strep.	4	33	D (3 days)	0	Circulatory Collapse. Laryngeal obstruction. Cerebral anoxia. Bullneck	Adrenal cortical damage (slight). Focal hemorrhages in heart and lungs.
27. F. H., 35226	70	?	−	E. Coli	6	78	D (17 days)	++ (With failure)	Bronchopneumonia. Laryngeal obstruction. Palatal Paralysis. Nephritis?	General and coonary Arteriosclerosis. Focal myocardial scaring. Lobular Pneumonia. Spotty necrosis and hyaline droplets in renal tubular epithelium.
28. A. S., 34636	5	+ (6 mos. & 2 yrs.)	+	−	3		R	+	Laryngeal obstruction. Pharyngeal Paralysis. Paralysis of legs.	

TABLE I—*Concluded*

NAME AND NUMBER	AGE	PREVIOUS IMMUNIZATION (AGE)	VIRULENCE TESTS[2]	OTHER BACTERIA (1)	DURATION (IN DAYS) PRIOR TO ANTITOXIN	N.P.N. (HIGHEST DETERMINATION)	OUTCOME (2)	CHIEF CLINICAL AND PATHOLOGICAL FINDINGS		
								Clinical Features		Significant Autopsy Findings (exclusive of local lesions)
								Cardiac abnormalities (3)	Other manifestations or complications	
29. E. M., 34094	6	0		−	14		D (12 hrs.)	+++	Cerebral thrombosis ?	Myocarditis. Thrombi in auricles and left ventricle.
30. J. W., 34743	5	0	+	α-strep.	3		R	++	Palatal Paralysis. Bullneck.	
Totals		12 positive	6 negative	β-strep. 14 cases	Average 4.7 days		D: 21 R: 9	27	Laryngeal or Tracheal obstruction: 15 Bullneck: 18 Circulatory Collapse: 11 Paralyses: 9	Myocarditis: 9 Adrenal cortical damage— Marked: 5 Slight: 2 Pneumonia: 7

[1] The six-figure numbers represent Johns Hopkins Hospital histories; the others are from Sydenham Hospital.

[2] Culture for C. diphtheriae was positive in each case. + = positive virulence tests; − = negative tests.

(1) Heavy growth unless otherwise stated. − = no significant growth.

(2) R = recovered; D = Died (in days after admission).

(3) Rough estimation of degree of abnormality as determined clinically and by Ekg.

(4) Diagnosis probable but not proved.

(5) Primary diagnosis, which was incorrect.

(6) Includes both necroses and "tubular degeneration" of adrenal cortical cells.

to touch, and which obliterates the normal curves between the mandible and the clavicle. This results in the so-called "bull-neck" appearance. This condition has been encountered so frequently during the recent epidemics, and has been associated so regularly with the most severe types of infection, that the term "bull-neck" diphtheria is often used to describe a particularly malignant variety. One wonders whether in these cases there may not be some strain difference of the infecting microorganism, which is not determined by the usual biological tests or whether other organisms may play a secondary role. Further studies should be done in hopes of clarifying these points, but routine cultures so far have not revealed secondary invaders that could be considered specific in producing this reaction. In the present series the bull-neck swelling was a prominent feature in 18 cases (60%).

Laryngeal or *tracheo-bronchial obstruction* was encountered in 15 (50%) of the patients and was nearly always relieved by prompt tracheotomy and suction. The suction could usually be accomplished successfully by rubber catheters but repeated bronchoscopic aspiration is often necessary. These patients show varying degrees of obstruction, with difficult wheezing respirations, cyanosis, and retraction of the interspaces or of the whole chest wall with inspiration. Signs of atelectasis or pneumonia also may be present. After tracheotomy pieces of membrane may be aspirated, and at times whole casts of the trachea or of the tracheo-bronchial tree can be obtained. Such procedures result in dramatic improvement in the patient's condition, but may need to be repeated many times. An indication of the success of this therapy is shown by the fact that atelectasis seemed to be an important contributing cause of death in only one of this group of patients, and in one other it was felt that tracheotomy may have been delayed too long, thus resulting in damage to the brain from anoxia. None of the deaths, however, could have been attributed entirely to laryngeal obstruction.

The Heart: The cardiac manifestations of diphtheria will not be discussed in any detail in this report as that aspect of the disease will be dealt with specifically in a study of a large group of patients from The Sydenham Hospital (6). Most patients who suffer from the severe form of diphtheria show evidence of myocardial involvement, and a high proportion of the deaths are due directly to this "complication."

In our group, 27 cases showed evidence of *myocardial damage*. This is usually determined—particularly in the sickest patients—on clinical grounds, but in others only electrocardiographically. The pathologic findings will be mentioned later. The myocardial damage may occur during any stage of the infection; in some it occurs early and as part of an overwhelming toxemia; in most it is found during the height of the disease; and in others it appears late, and the symptoms may develop suddenly when the patient seems well on the way to recovery. Many times it is accompanied by sudden circulatory collapse; this, however, is not always associated with demonstrable myocardial changes pathologically, as will be brought out later. The most reliable *clinical signs* of diphtheritic myocarditis are: a soft or weak first heart sound, particularly if a definite change has been noted in this sound from previous observation; an increase in heart rate; a fall in blood pressure; and in some cases enlargement of the liver. This latter finding is most commonly noted in young children. Arrhythmias of various types may be noted; extrasystoles and complete heart block being apparent from clinical grounds, and tachycardias such as ventricular tachycardia and auricular flutter usually being diagnosed by the electrocardiogram. Signs of congestive failure may occur in a few patients—if the patients survive long enough—but this is not a common finding. During the acute phase of the myocarditis in the patients studied recently at The Sydenham Hospital there was frequently found an increase in circulation time and sometimes a moderate increase in venous pressure. Further studies are being carried out along these lines, but one of us (S.C.), has observed enough patients with these findings to feel that they offer methods by which the diagnosis of myocardial weakness may be determined early and a means of following the efficacy of treatment.

The *electrocardiographic* changes are varied and not specific for diphtheria, but alterations from the normal tracing are observed so commonly—even in the milder cases—that the electrocardiogram offers us a useful test for diagnosis and prognosis of this complication. In the cases we are discussing the changes varied from the milder type, in which there was a slight alteration in the T-waves or increase in conduction time, to the most severe types such as complete heart block. The most significant findings in this group—with most patients show-

ing more than one abnormality—may be summarized roughly as follows: Lowering or inversion of T-waves was noted in 14 instances, depression of the S-T segments in 11, incomplete heart-block (including slight prolongation of the P-R interval) in 5, right axis deviation 5 times, and lowered voltage 5 times. Complete auriculo-ventricular block occurred 12 times and intraventricular block 9 times. Both of these were, of course, interpreted as evidence of advanced myocardial damage and as very grave prognostic signs, in most cases being found shortly before death. Ventricular tachycardia was diagnosed three times, auricular flutter twice, and auricular fibrillation once. In most of these patients, though it was felt very likely from clinical findings alone that myocarditis was present, the electrocardiograms were of great importance, not only in determining the degree of myocardial damage but also in following its trend from day to day or even from hour to hour.

Circulatory Collapse: It is well recognized that shock is a common occurrence in many types of acute infection, and this is particularly true of diphtheria. Rich (7) observed necrosis and "tubular degeneration" in the fascicular layer of the adrenals in many patients dying of severe acute infections including diphtheria, and speculated on its probable importance in producing circulatory collapse. There were 5 instances in the present series in which this pathological finding was noted in patients who developed sudden circulatory failure just before death, and it was felt that it may have played an important role in the fatal outcome. In one of these there was no microscopic evidence of myocarditis. However, the exact importance of this adrenal lesion cannot be definitely stated at the present time, and attempts at replacement therapy with adrenal cortical substance so far have not proved effectual, though the dosage employed may not have been adequate. Perhaps further studies on a larger series of cases will throw more light on this particular aspect of the problem.

The Nervous System: Paralysis of various cranial or peripheral nerves is a common accompaniment of diphtheria; the former occurring most often in the acute phase or slightly later, and the latter usually being delayed—sometimes as late as six weeks or longer after the acute attack. This late type of nerve lesion, which apparently has no analogue in any of the other infections in which neurotropic toxins or viruses are involved, must be due to early fixation of the toxin in the nerve cells and

then to very gradual changes in those cells, often taking weeks to develop to the clinical stage of peripheral neuritis. The recovery of the paralyzed muscles is slow, but usually complete. The earlier paralyses, which are most apt to involve the palatal muscles, are more serious complications because they usually occur when the patient is most acutely ill and make the problems of feeding and respiratory exchange more difficult during this critical period of the infection. This early type of paralysis is due to muscular damage, caused by local diffusion of the toxin, while a later type is a true nerve palsy and may come on three weeks or more after the acute onset.

As shown in the table, paralyses of one type or another were encountered in 9 patients in our group. In these cases there were 6 instances of palatal or pharyngeal paralysis; one each of ocular and diaphragmatic paralysis, and 4 instances of polyneuritis. Whereas all of these paralyses could be considered serious manifestations of the disease, the most dangerous ones were the palatal lesions, which occurred at the height of the infection in all except one patient and always in association with other complicating factors.

The Kidneys: While it is very common to find a moderate degree of albuminuria at one time or another in severe cases of diphtheria, a diagnosis of *nephritis* does not seem justified unless there are repeated findings of heavy albuminuria—and often casts as well—in association with elevation of the non-protein nitrogen in the blood. In 6 of our patients the diagnosis of nephritis was made on clinical grounds, but in none was it proved pathologically, as two of them recovered, no autopsy was done in another, and the other 3 showed only cloudy swelling, hydropic changes, and in one necroses and hyaline droplets in the renal tubular epithelium. Determinations of non-protein nitrogen in the blood were not made in every case, but the table shows that it is frequently elevated considerably even without definite evidence of nephritis. This must be due in part, at least, to temporary renal insufficiency in association with myocarditis and impaired renal circulation, but other factors may play a role, and preliminary determinations have shown that the electrolyte pattern may be very much altered in the patients who are seriously ill with diphtheria. Such findings may be related to damage to the heart or the adrenals rather than to a true nephritis.

(C) *Laboratory Findings: Leucocytosis:* The white blood cell count is usually stated to be only moderately elevated in diphtheria, but most of our cases showed a rather marked leucocytosis. In this series of patients the highest counts varied from 7,500 to 56,000, with an average of 20,600 per cubic millimeter. Many of the high values may have been due to the fact that other bacterial throat infections were frequently superimposed on the diphtheria—as shown by the culture reports. No definite correlation was noted between the severity of the diphtheria and the height of the white blood cell count.

Bacteriological Studies: Cultures from the throat, nose or trachea were positive in every case for *C. diptheriae* and in some instances the organism was grown from each of these sites. The tendency for the organisms to remain localized rather than to penetrate more deeply into the tissues or to invade the lymphatics or blood stream is illustrated by the fact that, with one exception, these bacteria were never isolated from other tissues or from the blood. In the one exception, (patient T.L.), a positive culture was obtained from the heart's blood just after death. This strain of C. diphtheriae was reported as being "non-virulent" just as the throat culture had been in this typical and fatal case of diphtheria. It is probable that this represents a terminal invasion of the blood stream, but it is possible that positive blood cultures might be obtained more often in the patients who are most seriously ill. Further indication that the diphtheria bacillus may, in an exceptional instance, invade the blood stream is afforded by a recent case report of a patient with bacterial endocarditis that was shown to be due to C. diphtheriae (8).

It is also of interest to note that in our series the routine *virulence tests* resulted in reports of "avirulent" organisms in 6 of the cases. In one patient the strain was found avirulent in one laboratory and virulent in the other, and in another the organism recovered from the trachea was found virulent and that from the nose avirulent. In none of these patients was there any question regarding the diagnosis of diphtheria. The methods employed in all these tests were the standard guinea-pig tests, which are done at times with the mixed culture, or more accurately with a pure culture. Ordinarily these are considered reliable tests for virulence, but it is obvious from this small series that they are not always accurate. Possibly other types of reactions, either

employing the rabbits' skin or the chicken in the experiment, may prove to be more reliable; or some other animal may be found in which the diphtheria toxin produces effects more closely parallel to its effects in the human being.

Other Bacteria: Various organisms other than diphtheria bacilli were isolated in the routine cultures of the nose, throat or trachea from most of the patients (as shown in the table). In most cases a heavy growth was obtained, making it appear that they may have been of some clinical significance in increasing the severity of the infection. This seems more likely in the case of *beta-hemolytic streptococci* than the others; this organism being recovered from 14 of the patients. It is because of the frequency of such cultural findings that antibiotic or chemotherapy is justified in hopes of modifying the infection, rather than for its effect on the diphtheria itself.

III. DIFFERENTIAL DIAGNOSIS

A few of the more common acute infections that frequently cause difficulty in diagnosis will be mentioned briefly.

(1) Acute tonsillitis or pharyngitis: The majority of these infections are caused by beta-hemolytic streptococci, and at times are very difficult to differentiate from pharyngeal diphtheria. If the pharyngeal lesion contains a confluent, membranous type of exudate, the diagnosis of diphtheria is very likely as the streptococcal infection usually results in a more patchy exudate which can be wiped off with a throat swab. The diphtheritic membrane is tough in consistency and is apt to leave a bleeding surface when scraped off. The patient with a streptococcal infection is apt to have a higher temperature than the one with diphtheria, and yet the latter usually looks sicker and is more apt to show signs of myocarditis. Smears and cultures should be taken from beneath the exudate and incubated immediately on Loeffler's medium and on blood agar. A positive smear for C. diphtheriae may be obtained from the Loeffler's medium in 8–12 hours. In a patient, however, in whom there is any suspicion of diphtheria, antitoxin should be given.

(2) Vincent's infection: In these cases the exudate is usually confluent and therefore hard to distinguish from diphtheria but the base has a tendency to ulcerate more deeply into the tissues. In these pa-

tients a smear taken from beneath the exudate will be positive for the fuso-spirochetal organisms characteristic of the infection. In a doubtful case a presumptive diagnosis of diphtheria should be made and the patient treated accordingly. Likewise, it is well to bear in mind that in an occasional case both infections may occur at the same time, and the diagnosis of diphtheria may be missed when that of Vincent's angina is established.

(3) Infectious mononucleosis: It is a fairly common occurrence for patients with the "anginal" type of this disease to be diagnosed diphtheria on the basis of the appearance of the throat. However, the correct diagnosis is usually made by the finding of enlargement of glands other than the local ones, a palpable spleen, and abnormal lymphocytes in the blood smear. The heterophile agglutinins appear in the blood in significant titers after ten days to two weeks and help to settle the diagnosis. We were interested to find several examples, however, of patients in whom *both* infections were present simultaneously. One of these patients, who was seen at The Johns Hopkins Hospital, showed all the characteristic features of infectious mononucleosis, and was treated as such for 10 days before the presence of diphtheria was determined. He continued to have fever and a membranous lesion in the throat from which positive cultures of C. diphtheriae were obtained only after the third attempt. The diphtheritic throat infection cleared up rapidly after antitoxin was administered. Two similar cases were observed within the past two years at The Sydenham Hospital, but in them the correct diagnosis of diphtheria was made on admission and they were treated at once with antitoxin. We have not encountered any similar report of the co-existence of these two infections, but believe that more cases will be found if the possibility is considered more often.

(4) Laryngo-tracheo-bronchitis and "croup": A child with a typical attack of croup may be no problem in diagnosis, because there is often a history of previous attacks with mild respiratory infections, with the symptoms usually developing at night. Moreover, though there may be considerable respiratory difficulty due to laryngeal edema, the child usually does not have much fever and may not seem very sick. However, there is a type of non-diphtheritic laryngitis, often associated with tracheitis and bronchitis, in which the child has

high fever, and may rapidly become seriously ill due to the acute infection and to respiratory obstruction. Such infections are usually caused by beta-hemolytic streptococci, pneumococci or H. influenzae and may be very hard to differentiate from diphtheria. The diagnosis may be made by examination of the throat and the larynx, and by cultures. However, it is often necessary to give diphtheria antitoxin when the diagnosis remains in doubt rather than wait for the cultures. Many times these patients with laryngo-tracheo-bronchitis require tracheotomy, which with antibiotic or chemotherapy may be life-saving.

(5) Peritonsillar Abscess: In some cases of pharyngeal diphtheria the inflammatory edema is intense and localized enough to cause the tissues to bulge forward in the nature of a peritonsillar abscess. Consequently many cases are so diagnosed and the suspected abscess is either incised or aspirated. This is a dangerous procedure in diphtheria, as the local situation is made worse rather than better inasmuch as no pus is released, and particularly because large amounts of toxin may be spread into the lymphatics and bloodstream in this way. Consequently, in any doubtful case, if it seems absolutely necessary to incise what seems to be a peritonsillar abscess, the patient should be given a large dose of diphtheria antitoxin first. This situation is illustrated by the case of J. K., (#13 in the table) as will be mentioned later. Another patient, a 10 year old girl (#3 A. F.) showed an extreme degree of peritonsillar swelling with bulging and there was a membrane on both tonsils. Those who first saw the patient felt that an abscess was present and that it should be incised or aspirated. It was decided, however, that the whole process could be explained on the basis of diphtheria and this decision was borne out by the fact that the throat lesions responded well to antitoxin. Similar situations occur fairly frequently in outbreaks of diphtheria, so that any case of suspected peritonsillar abscess should be examined critically from this standpoint and treated as diphtheria unless proved otherwise.

(6) Other Conditions: In some patients with diphtheritic myocarditis the condition at times is confused with *acute rheumatic fever*, and in the absence of joint pains the differentiation may present some difficulties. This is particularly true of the cases in which the myocardial changes are not severe and where there is no definite evidence of valve

lesions. At such times the diagnosis rests on careful examination of the local lesions and on repeated throat cultures in suspicious cases.

The early nerve lesions appearing during the height of the infection seldom present much of a diagnostic problem. However, the *peripheral neuritis* which usually has a delayed onset is often hard to differentiate from other types of neuritis. In these cases the primary infection may have been rather mild, and frequently the diagnosis of streptococcal pharyngitis had been made without taking cultures. In such patients the correct diagnosis is only suspected in retrospect, but may be supported occasionally by the persistence of positive throat cultures and residual changes in the electrocardiograms. The sequence of swallowing difficulty, loss of accommodation for near vision, then peripheral weakness—usually without paresthesias—is characteristic. In many cases the spinal fluid proteins are elevated, with no or only slight increase in cells. In this aspect, as well as in the character of the peripheral neuritis, diphtheritic polyneuritis may resemble the Guillian-Barré syndrome very closely.

IV. TREATMENT

The therapeutic problem in diphtheria involves three main categories: (a) neutralization of the circulating toxin as rapidly as possible, (b) relief of obstructive symptoms and (c) maintenance of an efficient circulatory system. As soon as the diagnosis of diphtheria is suspected, the patient is tested for sensitivity to horse serum, and if the tests are negative, the antitoxin is given at once. If the tests are positive the antitoxin is given in small and increasing doses at intervals of approximately one-half hour until the total amount has been given; the usual precautions are, of course, observed to deal with possible serum reactions. The usual method of administration is intramuscular for patients who are moderately ill, and intravenously for those whose infection is more severe. In most cases the total dose is given in one injection, and though there are no accurate methods of determining the exact amount needed, the plan is to be sure that an *excess* of antibodies is gotten into the blood as quickly as possible. In this group of severly ill patients the dosage varied from a low of 40,000 units to a high of 220,000 units, with an average of 120,000. Except for one patient, who received 40,000 units, the minimum dose was 80,000—even

in the case of small children. The one patient (J. K. #13), who was given the small dose, had been treated late because he had been diagnosed in another hospital as having a peritonsillar abscess. The diagnosis of diphtheria was therefore delayed because of this and he received specific therapy 17 days after the onset. The result in his case was death from myocarditis 65 days after admission, and the heart muscle showed extreme changes with destruction of cells, round-cell infiltration and fibrosis. In all the other patients the dosage of antitoxin was equal to or well above what is ordinarily considered adequate, though the tendency recently has been to give larger amounts. The average in this series of 120,000 units is quite high. This is the result of selection of the cases for their severity and the tendency to give larger doses to the more seriously ill patients. It is interesting to note, however, that in spite of this probable excess of antitoxin, the progress of the myocarditis in many of these patients is not obviously altered. In such cases the toxin presumably has been fixed by the cells of the myocardium early in the course of the infection. These tissues then may either go through a gradual or rapid process of cellular damage in spite of the antitoxin which almost certainly does not penetrate into the cell itself. Larger amounts of antitoxin would not help in such cases, but it is important to be certain that there is an excess of antibodies in the blood as long as toxin is being formed at the site of the local lesion. We will know more about the adequacy of such therapy when determinations of antitoxin levels in the blood have been made on a series of cases after specific treatment. These blood levels should be followed for several days in order to be sure of an adequate titer over the period of the acute infection.

The problem of dealing with obstructions in the various parts of the respiratory tract has been mentioned earlier and will only be summarized briefly. The wide experience at The Sydenham Hospital of Dr. Horace Hodes, Dr. Margaret Smith and others has served to emphasize the importance of *early* tracheotomy, before cerebral anoxia has developed and before the child has become too fatigued from severe and prolonged respiratory effort. Aspiration by suction—or particularly in those whose obstruction is lower down and who develop atelectasis—by bronchoscope, often produces dramatic improvement. An oxygen tent is frequently necessary, and it is important that the atmosphere

in the tent be saturated with water vapor and preferably kept at body temperature.

In treating derangements of the circulatory system in diphtheria one is often faced with a very difficult problem, one which does not have a parallel in any other condition. The reason for this is that circulatory failure may develop early in the course of an overwhelming infection with clinical and electrocardiographic evidence of severe myocardial damage; it may develop late, when the patient seems well on the way to recovery and in these cases also is usually associated with cardiac abnormalities; or circulatory collapse may occur during any phase of the acute infection and at times may not be explicable on the basis of myocardial damage. This problem of therapy will not be dealt with in any detail here, but it has been found that digitalization is not only a safe therapeutic procedure when carried out carefully but one that may be of real benefit, even during the acute phase of myocarditis, and is not contraindicated by signs of severe damage to the conduction system (9). The treatment of this "shock", when fully developed, is very difficult and on the whole has been unsatisfactory in the patients who are critically ill. Oxygen is given and a cautious attempt may be made to support the circulation with intravenous fluids, and thus to tide the patient over the most acute phase of the illness. Other substances have been tried—such as fifty per cent glucose, ascorbic acid and adrenal cortical hormone—but without evidence of any real benefit. This whole subject is being reviewed more completely in the other communication (6).

Penicillin was given intramuscularly in most of these patients during the past 2 years or more. In spite of the fact that the diphtheria bacilli are sensitive to penicillin, it was not found that the diphtheria itself was altered by its use; the probable explanation being that there is no evidence of its having any effect on the toxin. The chief purpose of the penicillin therapy was an attempt to prevent or eliminate other bacterial infections, and possibly to decrease the amount of toxin produced, by a direct effect on the diphtheria bacilli themselves.

V. PATHOLOGICAL FINDINGS

Autopsy data have been examined in 13 of the 21 patients who died. In 8 of these there was dilatation of the heart which was usually de-

scribed as marked. There was microscopic evidence of *myocarditis* in 9; this was often very extensive and involving all chambers of the heart, at other times of mild degree. In this respect the predominant changes varied from cellular infiltration to necrosis of myocardial fibers and finally to extensive areas of fibrosis. In 2 cases no lesions of myocarditis could be found, though on clinical grounds they were thought to have definite evidence of myocardial damage. In one of these patients—to be described later—there were advanced electrocardiographic abnormalities to support this opinion, and yet at autopsy the only microscopic changes noted in the myocardium were widespread petechial hemorrhages. One wonders whether it may not be true that in such cases the duration of the presumed myocarditis was too short to lead to changes which were visible microscopically. It is possible likewise that the petechiae may have played a role in production of the cardiac abnormalities. Lobular pneumonia was found in 4 cases, purulent bronchitis in 3 and some degree of atelectasis in 2. The local membranous lesion was observed in 7 instances and in the rest it had apparently disappeared and the local ulcerations healed. None of the patients showed a true "diphtheritic nephritis". The *adrenals* were involved in 7 cases, and in 5 of these the lesions were quite marked. These changes consisted of necroses and "tubular degeneration" in the fascicular layer of the cortex as had been described previously by Rich (7). The lesions in these patients were likewise either found or verified by Dr. Rich. They may be significant in helping to explain the occurrence of circulatory collapse and death.

VI. CASE REPORTS

The following two cases are summarized in order to emphasize some of the points that have been discussed:

Case 1. (*K. W.* *#24 in the table*): This patient was a white girl of 17 who was admitted to Sydenham Hospital on March 19th, 1947. She had never received diphtheria toxoid. About one month before admission she developed an upper respiratory infection which cleared up except for a cough which had persisted. Three days before admission she awoke with a sore throat, malaise, and anorexia. She was seen by her doctor who prescribed two pills every three hours. She vomited several times that day. Two days before admission she felt worse, had fever and severe sore throat. Her doctor returned and painted the throat with

some white liquid. That night the neck was noted to be swelling. On the next day the condition was much worse; she had great difficulty in swallowing and had a very painful neck. The diagnosis of diphtheria was then made; she was given 40,000 units of antitoxin and referred to The Sydenham Hospital. On admission the temperature was 101.2°, pulse 114, respirations 28, and blood pressure 120/84. She was acutely ill, had a bull-neck type of cervical swelling and was hoarse, but there was no marked respiratory distress and no cyanosis. The pharynx was extremely edematous, the tonsils being greatly swollen and covered by a grayish-white membrane which extended back over the posterior pharyngeal wall and up over the uvula. There was marked edema of the tissues of the neck with tenderness to palpation. The lungs were clear and the heart was normal in rhythm and in the character of the sounds. The urine showed a trace of albumin which two days later was 2 plus and on the 8th hospital day 4 plus. The white blood count on admission was 13,000 and on the 10th day had risen to 25,000. The throat culture was positive for virulent C. diphtheriae and for beta-hemolytic streptococci. An N.P.N. on the day before death was 60 milligrams per cent.

The patient was put in a steam tent and given 60,000 units of diphtheria antitoxin intravenously and 40,000 units intramuscularly. Penicillin was given in an initial dose of 60,000 units and then 30,000 units every 3 hours. She was extremely sick the first 4 or 5 days, but then seemed to be improving. The edema of the neck cleared gradually and at the time of death was entirely gone. The membrane in the throat also gradually disappeared. The blood pressure and heart sounds were normal during the first 4 or 5 days. On the 4th day she developed pharyngeal paralysis, and because of this, fluids were given intravenously and very carefully; frequent observations of the venous pressure on these days showed a moderate elevation before fluids were given and a considerable rise immediately afterward. On the 6th day she had bigeminal cardiac rhythm and the liver was down three finger-breadths below the costal margin. Digitalization was begun at that time, using the calculated dose of digalen intramuscularly over a period of 36 hours. During the first few days the electrocardiogram had shown only minor changes suggesting myocarditis, but on the 7th day there was evidence of very marked damage with inverted T-waves, bigeminal rhythm, and bundle branch block. On the 8th day she had improved, could swallow without regurgitation, and the heart sounds were better, though the first sound was definitely soft in character. The liver was no longer enlarged and the venous pressure normal. On the 9th day the blood pressure fell to 95/75, the patient had developed a striking pallor, and the electrocardiogram still showed marked myocardial damage. On the 11th day the blood pressure was 76/64; she looked very weak and listless, and the first heart sound was extremely weak and the circulation time prolonged. On the 12th day—the day of death—the temperature rose to 101° and she complained of joint pains. On that afternoon the blood pressure became unobtainable and the apical rate was counted at 200. An electrocardiogram at this time showed ventricular tachycardia. She was given quinidine intravenously, also plasma and was

put into an oxygen tent. The respirations became more and more difficult and she died one hour later.

At *autopsy* it was found that all the chambers of the heart were greatly dilated and the heart muscle appeared slightly paler than normal. It was of interest to find that all local traces of diptheria had cleared up, and this was noted both grossly and microscopically. Histologically there was found to be an extreme degree of myocarditis and widespread necroses of myocardial fibers; these changes were present in all chambers of the heart. Old necroses were found in the renal tubular epithelium which were interpreted as possibly being due to sulfonamide therapy, (this may have been administered before her admission but only in small doses and for a short time). One adrenal was atrophic, due to the presence of an angioma which was thought to have interfered with the circulation. In the other, there were necroses in the fascicular layer of the cortex. The anatomical diagnosis was: Diffuse myocardial necrosis and myocarditis. Necroses of renal tubular epithelium. Slight pulmonary edema. Angioma of right adrenal with atrophy, and necroses in the cortex of the other adrenal.

In summary this was a patient whose acute illness began rather gradually without seeming very serious until the third day. During this time the correct diagnosis was not suspected, though a bull-neck swelling had begun by the second day. Following admission her condition rapidly became worse; myocarditis developed with evidence of myocardial failure. She then seemed to respond to therapy and for a while her condition was improved. This was only temporary, however, for in spite of the fact that she had received antitoxin at the end of the third day of her illness, there was evidence of progression of the myocardial damage until the time of her death 11 days after admission. The clinical features could be explained by the extensive and fully developed changes of myocarditis, as well as the adrenal cortical damage, which may have played an important role in the final circulatory failure.

Case 2 (J. G. #6): This patient was a 35 year old white man who was admitted to The Johns Hopkins Hospital on March 25th, 1946, and died two days later. About one week before admission he had a mild earache which cleared up after local application of some drops. Two days before admission he developed a sore throat which rapidly became worse. A liquid medicine was given for this without relief and then penicillin lozenges were prescribed. The night before admission the sore throat was much worse and the neck became swollen. This swelling was much more marked the next morning and there was difficulty in breathing. He was brought to the hospital in an ambulance. On admission his temperature

was 103.4°, pulse 108, respirations 18, and blood pressure 90/65. He appeared acutely and seriously ill; there was a bull-neck swelling from the jaws to the clavicles, and respiratory difficulty was quite marked, but there was no cyanosis. There was a thin yellowish, bloodstained discharge from the nares. The pharynx itself could not be seen because of enormously swollen, edematous tonsils and peritonsillar tissues, the surface of which had a clear, shiny appearance. The airway was almost completely obstructed by these swollen tissues and no exudate was seen. The patient was expectorating bloody, thin, purulent material. The whole neck was greatly swollen and was red, indurated and tender. The lungs were clear except for distant breath sounds. The heart was regular and the sounds were considered normal. Inasmuch as no exudate and no membranous lesion was seen, the condition was thought to be a streptococcal infection and he was started on penicillin, 80,000 units every 3 hours, hot saline throat irrigations, and steam inhalations. The urine on admission showed a one plus reaction for albumin and many white blood cells and cellular casts. On the second day the albumin was 3 plus and there were many cellular casts. The white blood count was 17,400 on the first day. The first throat culture was considered negative for diphtheria bacilli and for beta-hemolytic streptococci, but another one taken the same day was reported positive for C. diphtheriae. This strain was found to be virulent. Because of the cervical cellulitis it was hoped that tracheotomy could be avoided, but 6 hours after admission the airway seemed to be nearly obstructed and an emergency tracheotomy was performed. This resulted in a temporary improvement, though the swelling of the neck seemed to be increasing. The pulse was then 120–130 and the respirations 30–35. At that time he was seen by the medical resident, Dr. Newman, who suspected diphtheria; 80,000 units of antitoxin were given intramuscularly and intravenous fluids were administered. The blood pressure had dropped from the previously low level to 66/30. An electrocardiogram taken that night showed marked depression of the S-T segments in leads I and II with elevation in leads III and IV; QRS was splintered in lead IV and T4 was notched. These changes were interpreted as showing widespread myocardial damage which could be due to diphtheria. Intravenous fluids were given slowly and he was also given 50% glucose and 300 mgms. of ascorbic acid intravenously. During the next day the patient became steadily worse, the temperature remaining at about 104°, and the pulse dropping to 80. At this time complete heart block was suspected and verified electrocardiographically. During the second night the pulse and blood pressure could not be obtained, the respirations fell to 10 per minute, and the heart rate to 68. The respirations continued to decline, cyanosis increased, and in spite of oxygen and coramine, respirations and heart stopped, death taking place just 36 hours after admission and three and one-half days after the onset of the acute illness.

The *autopsy* showed little on gross examination except dilatation of the heart, some small areas of consolidation in the lungs, and edema of the tissues of the neck. On microscopic examination, patches of diphtheritic membrane were found on the

trachea; there were hemorrhages and edema in the tissues of the neck, and widespread petechial hemorrhages in many organs. The myocardium was not considered abnormal on section except for widespread petechiae, but the adrenals showed very extensive changes; there was a broad band of necrosis in the fascicular layer that extended throughout the cortex. Dr. Rich felt that it was very likely that the adrenal lesions were related to the circulatory collapse which had been a prominent feature of the patient's illness.

The anatomical diagnosis was: Diphtheria of tonsils, trachea and pharynx. Edema and hemorrhages in the soft tissues of the neck. Hemorrhages in cervical and hilar lymph nodes. Petechial hemorrhages in skin, conjunctivae, pleura, mediastinum, epicardium, myocardium and stomach. Necrosis of the fascicular layer of both adrenals. Foci of lobular pneumonia.

In *summary* this patient had a fulminating and "malignant" form of diphtheria, characterized by a markedly edematous swelling of the tonsils, pharynx and neck; respiratory obstruction requiring tracheotomy, a delayed diagnosis due to the absence of visible membrane, and rapid progression to a fatal termination in spite of antitoxin. There was clinical evidence of myocardial damage which, except for the petechiae, was not apparent on microscopic section, possibly because of its short duration. There were extensive lesions in the adrenals which may have played a role in the fatal outcome.

VII. DISCUSSION

As was brought out earlier, diphtheria has shown a marked increase in prevalence in the past few years. This rise lasted for about 3 years in Baltimore and the vicinity, reaching its peak in 1946; during the past year there has been a definite decline again. It is probable that the incidence will decrease for a few years, then rise again as new outbreaks occur.

In view of the widespread nature of the lesions associated with this infection, it is not hard to understand the frequency with which the diagnosis of the primary condition is overlooked. However, if the possibility of diphtheria is kept in mind always in cases of acute respiratory infections, and if the local lesions are observed and cultured carefully, most cases will be recognized at an early stage. The great importance of immediate therapy with antitoxin is brought out in a striking manner by a consideration of the histories of the patients whose cases we have summarized, as well as by those of many more

which have come under observation. The development of a severe form of the disease, with evidence of extensive myocardial damage and often death, seems more closely related to delay in diagnosis and treatment than to any other factor, though the difference in strains and in susceptibility of the host must of course play an important role. If the toxin is not neutralized in the early stages of the infection it is evidently taken up by certain cells, and following this, damage to the cells may progress in spite of the fact that antibodies are present in the blood stream.

The data here presented also illustrate the fact that the clinical manifestations of the severe form of diphtheria may be extremely varied. The majority of these clinical findings may be correlated with and explained on the basis of damage by the toxin to such organs as the heart, the nervous system, or the adrenals. In addition, there may be serious respiratory complications resulting from the local membranous lesions. The cervical edema producing the bull-neck swelling has not been found to be due to bacterial invasion, and at autopsy no evidence of localized infection has been found in the involved tissues. It would seem that this reaction is most likely caused by local spread of the toxin.

It is our hope that this report may stimulate further inquiry into such problems as the adequacy of present immunization procedures. Studies are needed to determine the amount of antitoxin necessary to guarantee an excess in the blood as long as there is circulating toxin. Another interesting problem concerns the mechanism of the toxin's intracellular action. The fact that damage to susceptible cells develops so slowly to the point of clinical recognition—and even more slowly to obvious pathologic changes—suggests that the metabolism of the cells is altered by the diphtheria toxin in a very subtle but progressive manner. These alterations in the metabolic processes at first do not interfere with the cells' normal function to a detectable degree, but when fully developed and sufficiently widespread throughout the involved organ, functional deficiencies become apparent.

The chief purpose of this report is an effort to make the medical profession more alert toward the common occurrence of diphtheria in any community, and in all age groups, whether there is a history of previous immunization or not. Mild cases of the infection are often overlooked

and may get well without specific therapy. On the other hand, in patients who have a more severe type of the disease, any delay in treatment may result in a prolonged and complicated illness or in death. In those with the fulminating or malignant form of diphtheria the early administration of antitoxin is an emergency measure; otherwise such cases progress rapidly to a fatal termination.

VIII. SUMMARY

(1) The incidence of diphtheria has shown a decided rise in the past few years, and the severe form has been quite common. (2) The present report deals with the clinical and laboratory features observed in 30 such patients; the object being to illustrate the chief manifestations of the severe or malignant type of the infection. (3) There was clinical evidence of a high incidence of myocardial involvement; this was usually, but not always, verified in the cases in which autopsies were done. (4) Twelve of the 30 patients had a previous history of immunization against diphtheria. Similar experiences have been reported in other outbreaks. (5) The inaccuracy of the virulence test as routinely employed is indicated by the fact that 6 of the 30 strains of diphtheria bacilli isolated were reported as avirulent. (6) The average duration of the acute infection from its onset to the time antitoxin was given was 4.7 days. This delay in therapy was probably the most important cause of serious complications or death. (7) Stress is laid on the urgent necessity of suspecting the possibility of diphtheria in all questionable cases, so that the diagnosis may be made early and specific treatment administered promptly.

REFERENCES

1. Diphtheria Mortality in the Large Cities of the United States in 1946. 22d Report. J. A. M. A., **134:** 1540, 1947.
2. From Records of Baltimore City Health Department.
3. Eller, C. Howe, and Frobisher, Martin: An Outbreak of Diphtheria in Baltimore in 1944. Am. J. of Hyg., **42:** 179, 1945.
4. MacDonald, M. R.: The Diphtheria Situation in Cape Breton Island, Nova Scotia, over a two-year period. Can. J. Pub. Health, **37:** 391, 1946.
5. Fanning, J.: Outbreak of Diphtheria in a Highly Immunized Community. Brit. Med. J., **1:** 371, 1947.

6. ENGLE, M. E., MICHAEL, M., COBB, S., AND HODES, H. L.: Circulatory Changes in Diphtheria. (To be published.)
7. RICH, A. R.: A Peculiar Type of Adrenal Cortical Damage Associated with Acute Infections and Its Possible Relation to Circulatory Collapse. Bull. J. H. H., **74:** 1, 1944.
8. ZHEUTLIN, B.: Endocarditis Caused by Organisms Morphologically and Culturally Identified as C. Diphtheriae. Ann. Int. Medicine. (To be published.)
9. COBB, S., AND HODES, H. L.: Fluorescein Circulation Times in Diphtheria. (To be published.)

ANTIBODY FORMATION IN ALLOXAN DIABETES

A Comparison of the Precipitin Response to Egg Albumen of Normal Rabbits and of Rabbits with Alloxan Diabetes

TORRENCE P. B. PAYNE and ALAN H. CRUICKSHANK*

From the Department of Pathology, The Johns Hopkins University Medical School, Baltimore, Maryland

Received for Publication April 21, 1948

INTRODUCTION

It is generally accepted that patients with diabetes mellitus are more prone to infection than are non-diabetics, but the reason for it is unknown. Possible factors involved have been discussed by Rich (1) under the two following headings: metabolic alterations in the tissue fluids and the state of the mechanisms of resistance. As he points out, previous studies have been made upon human diabetics and totally pancreatectomised animals, in both of which it is difficult to achieve controlled investigation. Alloxan diabetes offered an opportunity to study the problem in animals with presumably normal exocrine pancreatic function and under controlled conditions.

The two main mechanisms of resistance to infection are the formation of antibody and the phagocytic activity of the leucocytes. In this study the first of these mechanisms, as estimated by the precipitin reaction, was investigated in alloxan diabetic rabbits.

EXPERIMENTAL PROCEDURE

Young adult male albino rabbits were made diabetic by the intravenous administration of alloxan (Eastman Kodak) in 4 percent solution in saline. Single doses between 100 mgm. per kgm. and 200 mgm. per kgm. were used. No insulin or glucose was administered at any time. Only those rabbits which maintained a blood sugar of about 300 mgm. percent (method of Folin and Malmros (2)), or over, with glycosuria were studied. At the time of testing for antibody formation the diabetes had been present for at least four months.

* Commonwealth Fund Fellow.

All the animals were fed the same diet—an unlimited amount of Purina Laboratory Chow and water.

Purified crystalline egg albumen, prepared from fresh hens' eggs by the method of Cole (3), was used as the antigen. The rabbits were immunized by four intravenous injections of a 1 percent solution of the crystalline egg albumen at 3 day intervals, 1 c.c. at the first injection,

TABLE I

EXPT I		Precipitin Test (Antiserum Dilutions)						Weight	Serum Proteins gms %			Blood Sugar	Urine	
Rabbit		1/2	1/4	1/8	1/16	1/32	1/64	kgm	Total	Alb	Glob	Mgm %	Glucose	Ketones
Diabetics	No. 7	+	+	+	+	0	0	2.5	5.8	4.2	1.6	348	++++	0
	8	+	+	+	0	0	0	2.9	5.8	4.2	1.6	352	++++	0
	9	+	+	+	0	0	0	3.6	6.1	4.5	1.6	343	++++	0
Normal Controls	21	+	+	+	+	0	0	2.7	5.8	3.8	2.0	90	0	0
	22	+	+	+	0	0	0	2.2	5.9	4.1	1.8	90	0	0
	23	+	+	+	0	0	0	2.5	5.3	4.0	1.3	100	0	0
	24	+	+	+	0	0	0	2.6	6.7	4.4	2.3	85	0	0
	25	+	+	+	0	0	0	2.0	5.5	4.3	1.2	85	0	0
	26	+	+	+	0	0	0	2.5	6.4	4.5	1.9	95	0	0
EXPT II														
Diabetics	A	+	+	+	0	0	0	3.75	5.6	4.4	1.2	308	++	0
	1	+	+	+	+	0	0	3.2	6.3	4.2	2.1	296	+++	0
	6	+	+	0	0	0	0	3.7	5.9	5.1	0.8	328	+++	0
Normal Controls	39	+	+	0	0	0	0	4.3	6.3	4.6	1.7	100	0	0
	40	+	+	0	0	0	0	3.5	6.4	4.8	1.6	95	0	0
	41	+	+	+	0	0	0	3.4	5.8	4.2	1.6	110	0	0

2 c.c. at the second, 4 c.c. at the third, and 6 c.c. at the fourth. Ten days after the last injection precipitin tests were done on the serum, using the antiserum dilution method (4). The optimum concentration of antigen for the test was first determined by titrating decreasing concentrations of antigen against undiluted antiserum. The highest dilution of antigen which gave a definite precipitate was chosen for the test. This was found to be $\frac{1}{300}$ of the original 1 percent solution.

Since several of our rabbits had lost weight and were weak, serum

protein determinations were made on all rabbits before immunization and at the time of the precipitin test. Total proteins were determined by the macro-Kjeldahl method (5), and serum albumen and globulin were separated by the method of Kingsley (6).

The diabetic animals were divided into two groups of three. The first group was compared with six normal controls (first part of Table I), the second group with three normal controls (second part of Table I).

RESULTS

Table I summarises the results of this experiment. In the case of the diabetic animals the blood sugar figures quoted are the average of five or six blood sugar estimations carried out during the period of the experiment. Only one serum protein estimation is quoted for each animal, for no significant differences were noted between the preliminary results and those obtained at the time of the precipitin test; the diabetic animals and the normals gave similar values on each occasion.

It will be seen that there is no difference in precipitin titre between the sera of the normal and those of the diabetic animals.

DISCUSSION

Alloxan diabetes in the rabbit is probably not comparable in every respect with human diabetes mellitus; for one thing the rabbit's metabolism is different from that of man, and this may be reflected in the fact that none of our animals ever became acidotic. At any rate, our rabbits presented continuous hyperglycaemia, glycosuria, polyphagia, polydipsia and polyuria. All of these findings are characteristic of human diabetes mellitus.

Depletion of serum proteins in the rabbit is known to cause a lessened capacity to produce agglutinin when compared with rabbits of similar age (7). In our animals, however, there was no detectable fall in serum proteins, although such a fall was considered quite possible, since several of the rabbits lost weight after they became diabetic.

It was felt that the precipitin test which we used was a sensitive enough index of antibody formation, since the finer differences which might be revealed by more elaborate methods and by hyperimmuniza-

tion would hardly have any practical bearing on the role played by antibody in the increased susceptibility of the diabetic to infections. The use of purified crystalline egg albumen obviated the possibility of any cross reactions which might have been encountered had a substance of more complex antigenic structure been employed.

From our results, therefore, it appears that in the rabbit the alloxan diabetic state by itself does not inhibit the production of antibody to crystalline egg albumen.

SUMMARY

In the absence of any depletion in serum proteins no difference in precipitin titre was found when normal and uncontrolled alloxan diabetic rabbits were immunized with purified crystalline egg albumen.

REFERENCES

(1) Rich, A. R.: The Pathogenesis of Tuberculosis, 1st edition, Charles C. Thomas, Springfield, Illinois, 1946.
(2) Folin, O. and Malmros, H.: J. Biol. Chem., **83:** 115, 1929.
(3) Cole, A. G.: Proc. Soc. Exper. Biol. and Med., **30:** 1162, 1932–33.
(4) Culbertson, J. T.: J. Immunol., **23:** 439, 1932.
(5) Campbell, W. R. and Hanna, M. I.: J. Biol. Chem., **119:** 1, 1937.
(6) MacClay, E. and Osterberg, A. E.: Proc. Staff Meeting Mayo Clinic, **15:** 600, 1940.
(7) Cannon, P. R., Chase, W. E., and Wissler, R. W.: J. Immunol. **47:** 133, 1943.

COMPARATIVE STUDY OF ANTIHISTAMINE SUBSTANCES[1]

I. Introduction and Dale Experiments

S. WALTER LANDAU

AND

LESLIE N. GAY[2]

Received for Publication April 26, 1948

Interest in recently introduced compounds which have been shown to counteract certain effects of histamine has found expression in a rapidly swelling literature on this subject. It has become increasingly difficult to analyze and compare the various reports on these compounds, which are being offered to the medical profession in steadily increasing number. Obviously many disagreements in the results of animal experiments are due to variations in the technic. Method, dosage, or time of exposure to the drug, these differ from author to author. The small number of animals used by some investigators; the lack of details supplied by others: these impair the value of other reports. The evaluation of results in patients is colored by the inevitable personal factor, because the allergic conditions for which these drugs are administered do not lend themselves to a gauge with which the degree of improvement can be reliably measured.

We attempted to compare a number of the antihistamine substances with uniform methods in a considerable number of animal experiments. In order to obtain an unbiased picture of their clinical value, the use of each drug was assigned to several members of our clinic group.

The solutions used for the animal experiments were made in our laboratory from the powdered drugs which we obtained through the courtesy of the various pharmaceutical houses. The French compound 3277RP was supplied through the kindness of Prof. Pasteur Vallery-Radot (Paris). For clinical use the drugs were available in capsules or tablets, furnished in generous amounts by the cooperating firms.

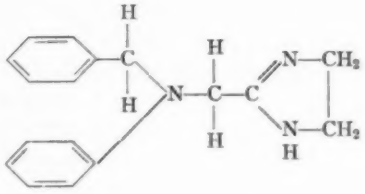
[1] This investigation has been aided by grants from G. D. Searle and Company, Eli Lilly and Company and the Houston Endowment, Incorporated.

[2] From the Protein Clinic of the Department of Medicine, The Johns Hopkins Hospital.

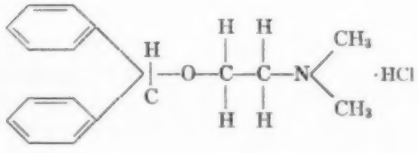
No data relative to the stability of these substances and their solutions have been published so far. Recently, however, one manufacturing company gave the information that the antihistamine drug they offer for clinical trial discolors with exposure to sunlight and interacts with rubber. In preliminary tests with the drugs, we found that the potency of higher dilutions of several drugs decreased considerably, even when these dilutions were kept in the refrigerator. In one instance, we found that 3277RP had oxidized en route from France. Detailed studies relative to the stability of the various antihistamine substances are urgently needed.

Various methods of testing these drugs in animals are available, and have been used. We believed that observation of their action on the intestinal strip of guinea-pigs and of their protective effects in guinea-pigs in vivo would give us a sufficiently representative picture of their comparative potency.

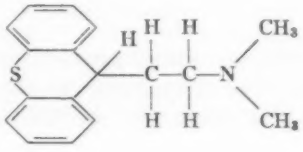
The compounds which we have used in the animal experiments are: Antistine (Ciba), Benadryl (Parke, Davis & Co.), 1721 (Searle & Co.), 3277 (Rhone Poulenc), Histadyl (Eli Lilly & Co.), Chlorothen (Lederle), Bromothen (Lederle), Pyribenzamine (Ciba), Neo-Antergan



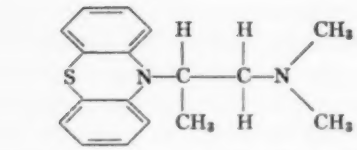
ANTISTINE



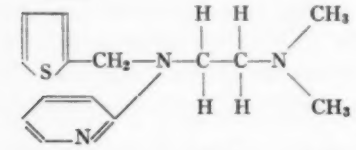
BENADRYL (Diphenhydramine HCl)



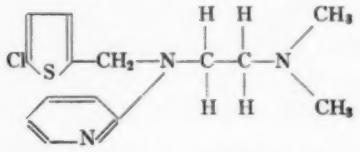
1721 (SEARLE)



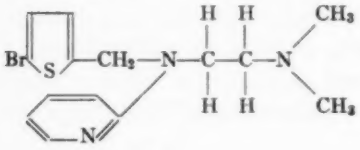
3277 R. P.



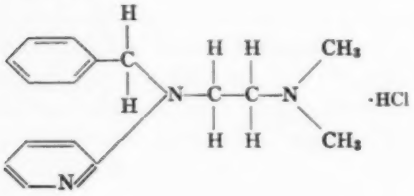
HISTADYL



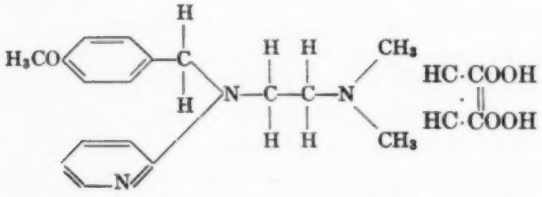
CHLOROTHEN



BROMOTHEN



PYRIBENZAMINE (Tripelenamine HCl)



NEO-ANTERGAN (Pyranisamine Maleate)

(Merck & Co.). (See structure formulas.) Antistine is a Swiss preparation; 3277 and Neo-Antergan are French compounds; all the other drugs have been developed in the United States. In the clinical trials

we used all these drugs except 3277, and, in addition, we used Hydryllin (Searle), a mixture of aminophyllin and diphenhydramine base, and 1913 (Searle), a mixture of aminophyllin and 1721 (Searle). In preliminary screening tests, we had also used two other Searle preparations, 1694, a xanthin salt of Benadryl, and 1695, a xanthin salt of the French compound 3015.

DALE EXPERIMENTS

(a) *Method:* The bath containing the suspended intestinal strip, along with a 3-liter flask containing Tyrode solution, was kept immersed in a 5-gallon battery jar. The water in this jar was agitated by a stirring motor, and its temperature was kept constant by an electric thermostat. Oxygen 95% with CO_2 5% bubbled through the bath by means of a gas disperser sealed in the bottom of the bath cylinder. Drainage was conducted through another opening underneath.[3] Most workers employ a bath temperature of 37.5° or 38° Centrigrade. Our observations seemed to indicate that a temperature of 36° Centigrade gave better working conditions. Frommel and co-workers (1) found a temperature of even 34° Centigrade optimal.

The Tyrode solution was prepared according to Burn (2), with the exception that solution B contained 0.25% $NaHCO_3$. We had had difficulties in maintaining the pH when we used Burn's original formula, and found this modification used by Halpern (3) more satisfactory.

In all our experiments, contraction of the strip was achieved by adding 10 gamma of histamine[4] to 100 cc. of the bath (0.1 gamma per cc.). In guinea-pigs of adequate sensitivity a maximal contraction is obtainable with this dose. When submaximal contractions resulted, the strip was discarded. We found, as other authors had found, that not only do guts of different guinea-pigs vary considerably in sensitivity but also segments of the same ileum vary.

Twenty years ago Kendall and collaborators published extensive

[3] We are indebted to Dr. A. H. Corwin (Chemistry Department, Johns Hopkins University) and to Dr. J. E. Cushing (Biology Department, Johns Hopkins University) for invaluable help and advice in setting up the apparatus.

[4] All doses of histamine are expressed as base. Histamine acid phosphate (Lilly or Burroughs Wellcome) was used.

studies on intestinal strips of guinea-pigs. Kendall and Varney (4) stated that their results "never became mathematical in their precision". They predicted that perfection would never be achieved until some of the idiosyncrasies of smooth muscle are better understood. This statement is equally true today, and should be kept in mind. On the basis of more than 600 Dale experiments, we learned to be extremely cautious in our evaluation of the tests. Recently, Chen and co-workers (5) have rightly stressed the erratic behavior of the gut in many instances.

In 1939 Anne-Marie Staub (6) pointed out that the effective concentration of the antihistamine 929F varied from one gut to another. She further observed that after suppression of the histamine reaction with 929F, washing out of the gut did not completely restore its activity. Even when the muscle contraction maintained the same intensity, such contraction could be more easily suppressed than before. According to Staub, subsequent tests are therefore not of equal value with the first test.

For several weeks, we worked with Staub's technic, i.e., *inhibition* of histamine contraction by preceding addition of the antihistamine to the bath, and experienced the following difficulties:

1. At times the histamine contraction achieved after repeated washing of the strip following the test would be higher than the contraction preceding the test. From which contraction should the percentage of inhibition be calculated?

2. With the more potent drugs, particularly Neo-Antergan, Chlorothen, and the slow-acting 3277, the gut failed indefinitely to regain its previous sensitivity to histamine. Therefore, we could not ascertain whether the gut had the same sensitivity during this test as that shown in the preceding histamine contraction.

3. In many instances we observed a two-phasic effect. When histamine was given after the antihistamine, there resulted a contraction to a certain height followed immediately by relaxation to a certain point. In such cases how should the degree of inhibition be calculated?

Because of these uncertainties we adopted Halpern's (3) technic, which estimates the degree of *relaxation* caused by the addition of the antihistaminic drug to the bath, after the histamine contraction has taken place. Using this method, we arrived at the same conclusion as

Schild (7): It is preferable to use a new piece of gut for each test. Washing the gut until the original height of histamine contraction is restored may involve as much time as suspending a new strip. If Staub's (6) observation is valid, even the same response to histamine as before does not guarantee the same sensitivity to the antihistaminic substance.

Therefore, the procedure finally chosen was as follows: after suspension of the strip, 0.1 gamma/cc histamine base was added to the Tyrode solution. If a maximal contraction was obtained, the strip

TABLE 1

Action of various antihistamine drugs on the histamine contraction (0.1 gamma/cc) of the intestinal strips of guinea-pigs

Exposure to histamine and to drug each one minute

DRUG	0.01 GAMMA/CC	0.02 GAMMA/CC	0.05 GAMMA/CC	0.1 GAMMA/CC	0.2 GAMMA/CC
Antistine	12	25	52	88	97
Benadryl	38	71	83	96	
1721	32	56	81	90	
3277RP	30	55	73	84	
Histadyl	93	97			
Bromothen	85	92			
Chlorothen	98				
Pyribenzamine	81	95			
Neo-Antergan	73	97			

NOTE: Numbers indicate percentage of relaxation.

was washed and a second histamine contraction elicited. One minute later the antihistamine compound was added to the bath. (Histamine, as well as its antagonist, was always contained in the same volume of distilled water, one cc.) After conclusion of the test, the strip was discarded and a new piece of gut suspended.

(b) *Results:* Table 1 shows the percentage of relaxation (from the histamine contraction) accomplished by varying amounts of each drug when the drum was stopped one minute after the drug had been added. No test was accepted for calculation of the percentage when the slightest doubt existed as to the normal sensitivity of the gut. Each number in the first column (0.01 gamma/cc) represents the average of eight to

twelve determinations, each number in the other columns represents the average of four to seven determinations.

Antistine proved to be of lowest potency. Benadryl, 1721, and 3277 had about equal moderate effectiveness, Benadryl's being slightly higher than the other two. Histadyl, Bromothen, Chlorothen, Pyribenzamine, and Neo-Antergan can be grouped together, for 0.01

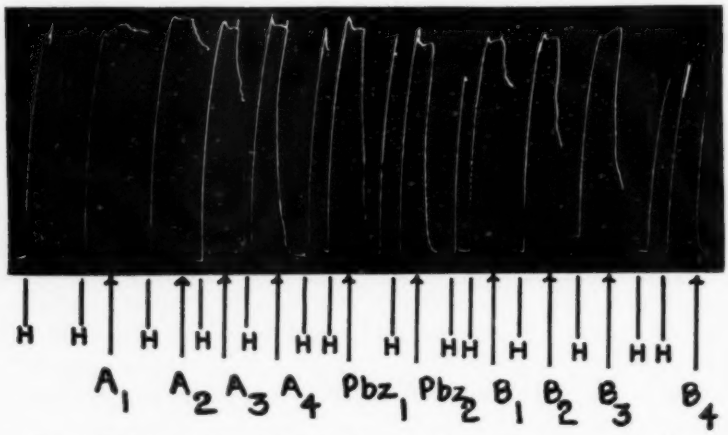


FIG. 1. Dale test, intestinal strip of guinea pig. Exposure to histamine one minute, to antihistamine substance one minute.

H = 0.1 gamma/cc histamine base	Pbz 1 = 0.01 gamma/cc Pyribenzamine
A1 = 0.01 gamma/cc Antistine	Pbz 2 = 0.02 gamma/cc Pyribenzamine
A2 = 0.02 gamma/cc Antistine	B1 = 0.01 gamma/cc Benadryl
A3 = 0.05 gamma/cc Antistine	B2 = 0.02 gamma/cc Benadryl
A4 = 0.1 gamma/cc Antistine	B3 = 0.05 gamma/cc Benadryl

B4 = 0.1 gamma/cc Benadryl

to 0.02 gamma/cc of these compounds caused almost complete relaxation of the contracted muscle within one minute. (Table 1, fig. 1 and 2)

Table 2 shows that the differences in the most potent group are insignificant. When we determined the time required to reach base line after addition of the antagonist, we found that the activity of the drugs in this group was very similar.

On closer analysis, the apparent superiority of Benadryl over 3277

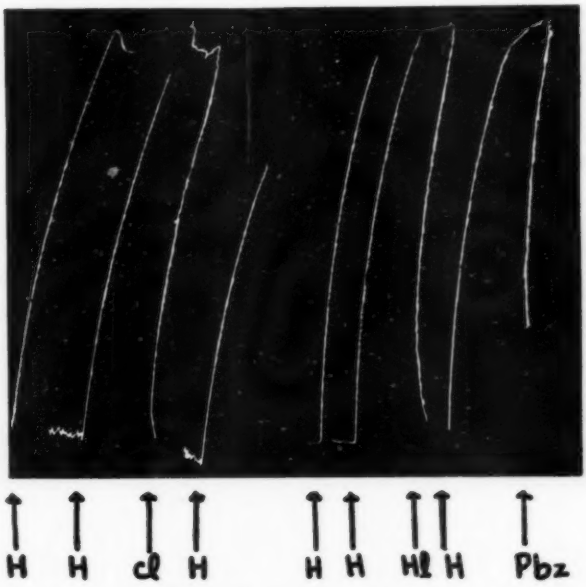


FIG. 2. Dale test, intestinal strip of guinea pig. Exposure to histamine one minute, to antihistamine substance one minute.
H = 0.1 gamma/cc histamine base Hl = 0.01 gamma/cc Histadyl
Cl = 0.01 gamma/cc Chlorothen Pbz = 0.01 gamma/cc Pyribenzamine

TABLE 2

Time required for 100% relaxation of the intestinal strip contracted with 0.1 gamma/cc histamine for 5 minutes

DRUG (0.01 GAMMA/CC)	TIME
	seconds
Histadyl	85
Chlorothen	105
Pyribenzamine	100
Neo-Antergan	90

disappeared. The French preparation was found to have much slower action than the other compounds. After adding 3277 to the bath, from 30 to 40 seconds was required for relaxation to begin in contrast

with 5 to 8 seconds required by the other substances. Correspondingly, if the strip was exposed for 5 minutes to 3277, 0.01 gamma/cc achieved almost complete relaxation; whereas, the relaxation after a 5 minute exposure to Benadryl (0.01 gamma/cc) averaged 65%. In a five-minute exposure, the same dose of Antistine did not produce more than 42% relaxation. (Fig. 3a)

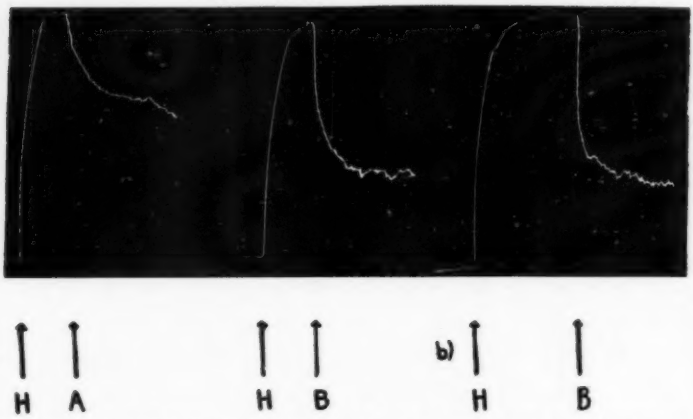


FIG. 3a. Dale test, intestinal strip of guinea pig. Exposure to histamine one minute, to antihistamine substance five minutes.

FIG. 3b. Same. Exposure to histamine five minutes, to antihistamine substance five minutes.

H = 0.1 gamma/cc histamine base
A = 0.01 gamma/cc Antistine
B = 0.01 gamma/cc Benadryl

When we allowed histamine to act on the gut for five minutes instead of one, the relaxation due to the following antagonist was not appreciably changed in any group. This is illustrated in Fig. 3b and Fig. 4.

(c) *Discussion:* Comparison of our results with results reported in the literature is difficult because of the lack of uniform procedure. Some authors used histamine diphosphate; others used histamine dihydrochloride. Certain writers express their histamine dose in terms of the salt, others express dosage in terms of histamine base; a few neglect to give this necessary information. It is confusing to find

figures of "histamine" concentration in the bath, tabulated without the author's taking account of the fact that the figures of one investigator represent histamine dihydrochloride but those of the second investi-

FIG. 4. Dale test, intestinal strip of guinea pig. Exposure to histamine five minutes, to antihistamine substance two minutes.

H = 0.1 gamma histamine base
Pbz = 0.01 gamma Pyribenzamine

gator represent histamine diphosphate (dihydrochloride contains 60% histamine base; diphosphate contains 36% histamine base.)

Table 3 is a compilation of quantitative data presented in sufficient detail to enable one to calculate the action of a single drug against histamine. The figures of the authors, given in terms of histamine salt, have been changed to histamine base values. Benadryl counter-

acted approximately an equal amount of histamine. Neo-Antergan and Pyribenzamine were effective in counteracting 6 to 10 times as much histamine. Histadyl was effective against 18 to 72 times as much histamine; however, this drug was allowed to act two minutes on the gut.

Data comparing the activity of several compounds are few and conflicting. Taking the average from 12 to 16 experiments, Winter (11) determined the inhibition for each of five drugs. He found this percentage to be: Neo-Antergan 81; Pyribenzamine 68; 3277, 45; Benadryl 42; Hetramine 11. Graham (13) found the quantity of various compounds required *to reduce by one-half* the spasm produced by

TABLE 3

Results in the literature of effective counteraction of histamine contraction in the Dale bath

AUTHOR	METHOD USED	HISTAMINE	ANTIHISTAMINE DRUG		TIME OF CONTACT
			Kind	gamma/cc	
		gamma/cc			
Loew et al. (8)	Inhibition	0.02 appr.	Benadryl	0.02–0.04	one min.
R. Mayer (9)	Inhibition	0.036–0.36	Pyribenzamine	0.02–0.04	one min.
Bovet et al. (10)	Relaxation	0.06	Neo-Antergan	0.01	?
Winter (11)	Inhibition	0.12	Neo-Antergan	0.02	one min.
Lee et al. (12)	Relaxation	0.18	Histadyl	0.0025–0.01	two min.

1 gamma/75 cc histamine to be: Neo-Antergan 0.07; Benadryl 0.7; Antistine 5.3. In his experiments the drug was allowed to act on the gut for two minutes. Lee and collaborators (12), who also exposed the gut for two minutes to the antagonist, found Histadyl (01013) on the average 4.9 times as active as Benadryl, but 0.8 as active as Pyribenzamine.

Most of our results are similar to those determined by Winter (11). When 0.01 gamma/cc was used, Neo-Antergan was twice as effective as Benadryl. We found 3277, if the effect in one minute is measured, in the same group as Benadryl. But in our tests, Neo-Antergan was somewhat less potent than Pyribenzamine. In marked contrast to Lee and collaborators (12), Histadyl in small doses and on short contact was more effective than Pyribenzamine.

(d) *Conclusions:* In view of the present uncertainties and the in-

evitable inaccuracies in this kind of determination, we feel that not much weight should be given to small differences. Conservatively, we can state these results: Within one minute, Histadyl, Bromothen, Chlorothen, Pyribenzamine, and Neo-Antergan completely counteracted five times as much histamine. Within one and a half to one and three-quarter minutes, these drugs counteracted ten times as much histamine. Within one minute, Benadryl completely antagonized an equal amount of histamine but Antistine was fully effective against only one-half the amount of histamine. The French preparation, 3277, differed from the other compounds in its slow action. *In five minutes* this drug was able completely to antagonize ten times as much histamine.

In the literature there are very few reliable data concerning the degree to which antihistaminic drugs counteract the contraction produced by the homologous antigen in a sensitized guinea-pig strip. Available data indicate that larger doses are required to be effective against antigen contractions than are required to be effective against histamine contractions. (Staub (6), LaBarre et Reuse (14), R. Meier (15).) Our preliminary tests, to be extended and published later, confirm these observations. Rose and collaborators (16) came to the opposite conclusion. They used strips from guinea-pigs passively sensitized, and no statement was made concerning the number of their tests.

SUMMARY

Our study of the action of 9 antihistamine substances in Dale tests revealed three groups of potency: Antistine was weakest; Benadryl, 1721 (Searle), 3277RP were moderate; Histadyl, Chlorothen, Bromothen, Pyribenzamine and Neo-Antergan were highly potent.

These values are based on one-minute contact of the smooth muscle with the drug. In prolonged contact 3277 equaled the effectiveness of the drugs in the most potent group.

REFERENCES

1. Frommel, E., Favre, M. A., Valette, F.: Arch. Internat. de Pharmacodyn. et de thérap., **73:** 355, 1947.
2. Burn, J. H.: Biological Standardization, London 1937.
3. Halpern, B. N.: Arch. Internat. de Pharmacodyn. et de thérap., **68:** 339, 1942.

4. KENDALL, A. J. AND VARNEY, P. L.: J. Infect. Dis., **41:** 143, 1927.
5. CHEN, G., ENSOR, C. R., AND CLARK, J. G.: J. Pharmacol., **92:** 90, 1948.
6. STAUB, ANNE-MARIE: Ann. Instit. Pasteur, **63:** 400 and 485, 1939.
7. SCHILD, H. O.: Brit. J. Pharmacol., **2:** 189, 1947.
8. LOEW, E. R., MACMILLAN, R., AND KAISER, M. E.: J. Pharmacol., **86:** 299, 1946.
9. MAYER, R. L.: J. Allergy, **17:** 153, 1946.
10. BOVET, P., HORCLOIS, R. ET WALTHERT, F.: Compt. Rend. Soc. de Biol., **138:** 991, 1944.
11. WINTER, C. A.: J. Pharmacol., **90:** 224, 1947.
12. LEE, H. M., DINWIDDLE, W. G., AND CHEN, K. K.: J. Pharmacol., **90:** 83, 1947.
13. GRAHAM, J. D. P.: J. Pharmacol., **91:** 103, 1947.
14. LABARRE, J. ET REUSE, J.: Compt. Rend. Soc. de Biol., **140:** 1039, 1946.
15. MEIER, R. AND BUCHER, K.: Schweiz. Med. Wochenschr., **76:** 294, 1946.
16. ROSE, J. M., FEINBERG, A. R., FRIEDLAENDER, S., AND FEINBERG, S. M.: J. Allergy, **18:** 149, 1947.

COMPARATIVE STUDY OF ANTIHISTAMINE SUBSTANCES[1]

II. Activity in vivo against Histamine Intoxication and Anaphylactic Shock of Guinea Pigs

S. WALTER LANDAU, HENRY J. L. MARRIOTT, AND LESLIE N. GAY[2]

A. HISTAMINE INTOXICATION

The antagonizing effect of the antihistamine substances against the bronchoconstrictor action of histamine in the intact guinea pig has been examined through various procedures. Staub (1), a pioneer in the field, injected a fixed dose of the antihistamine compound prior to the intravenous administration of histamine, and observed the number of lethal doses of histamine that the animals would tolerate. Subsequently, a number of investigators employed Staub's technique. Halpern (2, 3), using this method, compared a large series of compounds, and with his latest preparation (3277) arrived at the figure of 1500 lethal doses of histamine against which 20 mg of 3277 would protect.

The same author inaugurated the testing of the drugs against the effect of aerosoled histamine. In a few experiments he gave the antihistamine compound per os; in others he mixed it with histamine in the aerosol; in most experiments he administered the antihistamine subcutaneously at various intervals before the exposure to histamine aerosol. This latter technique has been adopted by many investigators. Halpern (3) observed the time until the appearance of severe asthma, and considered those animals who had no symptoms after 10 minutes of inhalation as protected. Mayer (4) and Winter (5) also employed this technique to test reduction of or freedom from symptoms. A significant reduction in the mortality rate was taken as the indicator by other authors (Loew and co-workers (12), Litchfield and co-workers (6), Meier and Bucher (7), Lee and co-workers (8)).

The third method employed consists of determining the protection

[1] This investigation has been aided by grants from G. D. Searle and Company, Eli Lilly and Company and the Houston Endowment, Incorporated.

[2] From the Protein Clinic of the Department of Medicine, The Johns Hopkins Hospital.

created by prior administration of the antihistamine compound to resist one lethal dose of histamine injected into the bloodstream. Winter (5), as well as Rose and collaborators (9), has studied the action of histamine antagonists in this manner. Independently, we had chosen this technique before we started our comparative study. We believe that death from the minimal lethal dose of histamine as the indicator eliminates any subjective factor from evaluation of the protective effect, and that parenteral administration of drugs best guarantees the introduction into the animal of the dosage selected for the experiment, an all-important factor in quantitative studies.

(*a*) *Method:* Our aim was to determine the smallest dose of the various compounds that would prevent death from one lethal dose of histamine in 100% of the animals. This minimal lethal dose was again established as 0.5 mg/kg histamine base when injected intracardially (Landau and Gay (10)). The procedure was as follows: In a first set of experiments 0.5 mg/kg histamine was injected into the heart, thirty minutes after the subcutaneous injection of an antihistamine compound. In further series of experiments, the dose of this compound was increased or decreased depending on the percentage of surviving animals. With some drugs we could base our initial dose on reports in the literature, with others we had to find our way slowly to the protective dose.

Interested in the duration of the protective effect, we injected guinea pigs intracardially with one lethal dose of histamine two hours after the subcutaneous administration of that dose of the compound that had given 100% protection after half an hour. Those drugs that gave significant protection after two hours were chosen for determination of their effect after five hours.

All the animals were observed for severity of symptoms. In each case of death, an autopsy was performed. In a number of animals, the dissection showed considerable hemorrhage from the heart. We believed that in these cases the violent convulsions effected by histamine had caused bleeding from the site of the heart puncture;[3] consequently, these deaths were due to a combination of causes: intracardiac injection plus reaction to histamine. Therefore, we excluded

[3] Intracardial injection of salt solution produced no trouble in ten animals.

from the series those animals in which we found such a major hemorrhage. We had followed the same practice in our work on arginine and histidine. A survey showed that if these animals had been included, the total results would have lowered some of our figures of survival by 5% to 15%, but would not have influenced the order of potency of the different drugs.

(*b*) *Results:* Table 1[4] shows the degree of protection from varying doses of nine antihistamine compounds, injected one-half hour prior to the lethal dose of histamine. The minimal 100% protective doses are in mg/kg: Antistine 1.5, Benadryl 0.6, drug 1721 (Searle) 0.4, drug 3277RP 0.2, Bromothen 0.05, Chlorothen 0.05, Histadyl 0.05, Pyribenzamine 0.04, Neo-Antergan 0.02. The percentage of animals that show less than three-plus symptoms runs almost parallel to the increasing percentage of survival. When a multiple of the minimal dose that protected 100% of the animals was given, as in the case of Chlorothen and Bromothen, the animals remained practically free from symptoms.

Table 2 indicates the duration of the protective effect. After two hours no appreciable protection remained in the series with Antistine, Benadryl, and Histadyl; from 60% to 100% protection remained in the series with 1721, Bromothen, Chlorothen, Pyribenzamine, 3277 and Neo-Antergan. After five hours this remaining activity had completely subsided in the Chlorothen series and had become insignificant in the Pyribenzamine and Neo-Antergan series. However, 73% of the animals injected with 3277 survived one lethal dose of histamine injected five hours later.

(*c*) *Discussion:* The order of potency against histamine in these in vivo experiments parallels and confirms the results of our Dale tests in the following characteristics: Antistine requires the largest dose for protection; Histadyl, Chlorothen, Bromothen, Pyribenzamine and Neo-Antergan are effective in very small doses; Benadryl, 1721 and 3277, when we consider the protection within 30 minutes, occupy a middle place. In this middle group, Benadryl is definitely inferior to 1721 and 3277. In contrast to the Dale results Neo-Antergan emerges as the most potent drug, its minimal 100% protective dose being half

[4] The figures are tabulated as in Winter's (5) study to facilitate comparison.

TABLE 1

Protection of guinea pigs with antihistamine compounds, given subcutaneously, against one lethal dose of histamine, given intracardially 30 minutes later

ANTIHISTAMINE COMPOUND (SUBCUTANEOUSLY)		SEVERITY OF SYMPTOMS* (NUMBER OF ANIMALS					SURVIVAL %	LESS THAN 3+ SYMPTOMS %
Kind	Dose	0	+	++	+++	Death		
	mg/kg							
None (controls)		0	0	0	1	9	10	0
Antistine	0.01	0	0	0	0	10	0	0
(62 animals)	0.04	0	0	0	0	8	0	0
	0.5	1	1	1	2	5	50	30
	1.0	0	2	3	2	3	70	50
	1.5	0	1	4	8	0	100	39
	2.0	2	5	1	2	0	100	80
Benadryl	0.2	0	0	2	7	3	75	17
(43 animals)	0.4	0	1	3	4	2	80	50
	0.5	0	2	3	4	1	90	50
	0.6	0	3	4	4	0	100	64
1721 (Searle)	0.1	1	0	0	0	8	12	12
(45 animals)	0.2	0	2	0	1	9	25	18
	0.3	0	3	1	6	2	83	33
	0.4	1	2	2	7	0	100	42
3277 RP	0.1	0	1	2	5	2	80	30
(22 animals)	0.2	6	2	2	2	0	100	85
Bromothen	0.01	1	0	0	3	6	40	10
(73 animals)	0.02	0	2	5	5	2	86	50
	0.04	0	0	6	7	3	81	37
	0.05	0	4	0	7	0	100	36
	0.06	0	3	5	4	0	100	67
	0.2	9	1	0	0	0	100	100
Chlorothen	0.005	0	0	0	1	6	14	0
(93 animals)	0.01	0	0	3	12	1	93	18
	0.02	1	3	5	9	2	90	45
	0.04	1	5	4	6	2	89	62
	0.05	0	3	2	5	0	100	50
	0.06	0	3	3	6	0	100	50
	0.2	9	1	0	0	0	100	100
Histadyl	0.04	0	1	1	1	7	30	20
(41 animals)	0.05	0	1	5	4	0	100	60
	0.06	2	2	3	3	0	100	70
	0.1	4	4	1	2	0	100	90

TABLE 1—*Continued*

ANTIHISTAMINE COMPOUND (SUBCUTANEOUSLY)		SEVERITY OF SYMPTOMS* (NUMBER OF ANIMALS)					SURVIVAL %	LESS THAN 3+ SYMPTOMS %
Kind	Dose	0	+	++	+++	Death		
	mg/kg							
Pyribenzamine	0.02	0	0	0	5	7	42	0
(34 animals)	0.03	0	0	2	8	1	91	18
	0.04	0	1	4	6	0	100	45
Neo-Antergan	0.005	0	0	0	5	5	50	0
(32 animals)	0.01	0	2	2	6	1	91	36
	0.02	3	3	4	2	0	100	85

*0 = no symptoms; + = accelerated respiration, itching; ++ = cough, asthma; +++ = convulsions, weakness.

TABLE 2

Duration of protection with 100% effective dose of antihistamine compounds (subcutaneously) against one lethal dose of histamine (intracardially)

ANTIHISTAMINE COMPOUND	AFTER 2 HOURS			AFTER 5 HOURS	
	Number of Animals	Survival %	Less than 3+ Symptoms %	Number of Animals	Survival %
Antistine	7	14	0		
Benadryl	6	16	0		
1721 (Searle)	10	60	0		
3277RP	10	100	90	11	73
Bromothen	19	84	36		
Chlorothen	21	81	24	5	0
Histadyl	7	14	14		
Pyribenzamine	19	84	36	7	30
Neo-Antergan	19	100	47	4	25

as much as that of Pyribenzamine and less than half as much as that of the other drugs in the same group.

Winter (5), using the same technique with the exception that he gave histamine intravenously, tested six compounds, four of which are included in our study. He, likewise, found Neo-Antergan to be the most potent drug, followed by Pyribenzamine. His 100% protective dose of Neo-Antergan was 0.01 mg/kg. With this dose we had 91% survival, an insignificant difference, particularly so in view of the fact

that the percentage of animals with less than three-plus symptoms was almost the same in his and our series. Our figures for Pyribenzamine are slightly less favorable than Winter's. In this author's studies, 3277RP was definitely more effective than Benadryl; the dose for 100% survival, however, was not determined for these two drugs.

Figures for Histadyl have been published by Feinberg and Bernstein (11). They achieved 100% protection by intraperitoneal injection of 0.1 mg/kg, but only 50% protection with 0.05 mg/kg. Rose and co-workers (9) also injected the antihistamine substance intraperitoneally and administered the lethal dose of histamine as early as fifteen minutes after the protective agent. It may be due to these differences in procedure that in their studies as much as 0.1 mg/kg Neo-Antergan gave only 78% protection. With Benadryl, Rose and co-workers (9) still had, surprisingly, a mortality of 29% with a dose of 3 mg/kg.

When we compare our results with those of authors who used aerosoled histamine, we find that Sherrod and co-workers (12) in their studies determined the potency of Neo-Antergan to be four times and twenty times, respectively, higher than that of Pyribenzamine and of Benadryl. These results refer to intraperitoneal injection of the antihistamine substance and to its ability significantly to reduce the incidence of mortality. Their conclusion might appear as too unfavorable towards Pyribenzamine, for with 0.3 mg/kg of this drug their reduction of mortality was 65% compared with 20% reduction with 1.5 mg/kg Benadryl and 30% reduction with 0.075 mg/kg Neo-Antergan.

The comparatively weak activity of 3277 in our studies, as well as in Winter's, is surprising in view of the fact that Halpern found 3277 far superior to Neo-Antergan, when these drugs were used against multiple lethal doses of histamine. 3277 protected, as mentioned, against 1500 lethal doses, whereas Neo-Antergan protected against only 80 lethal doses. Winter tested the effect of both drugs against massive doses of histamine, but found no impressive difference; however, the number of animals used was small. Halpern's figures have not been confirmed by other workers in France. Lerman and Goldfeder (13) noted that the protection against aerosoled histamine seemed to be more complete with Neo-Antergan than with 3277.

As to the duration of protection, our results confirm Halpern's statement, made on the basis of aerosol experiments, that 3277 dis-

tinguishes itself by its prolonged effect. In our studies, this drug was the only one that had lost very little of its action within five hours. Litchfield and co-workers (6) found the duration of protection against aerosoled histamine shorter for Thenyl (Histadyl) than for Pyribenzamine. A corresponding difference between these two drugs was obvious in our series. These authors also found that with the same method, Chlorothen and Bromothen each gave protection for twice as long as did Pyribenzamine. We found that with these three drugs, the rate of survival was equal after two hours and no significant effect was present with either of the compounds after five hours.

B. ANAPHYLAXIS

(*a*) *Method:* In a large majority of our anaphylaxis experiments, sheep serum was used as antigen. A single sensitizing dose (0.5 cc.) was given intraperitoneally; the challenging dose (0.25 cc.) was given intracardially in most series, in a few series with Neo-Antergan the challenging dose was injected into the jugular vein. (See table 3.) With this technique 86% of the controls died from anaphylactic shock. In some series we employed egg white as antigen, giving 2 cc. of a 10% solution as sensitizing dose and 0.5 cc. of a 2% solution as challenging dose; all these controls (12) died in anaphylactic shock. In table 3 all figures without an asterisk denote sheep serum as the antigen. Twenty-one days after sensitization, the challenging dose was injected into all animals. The antihistamine drug was administered, subcutaneously, thirty minutes prior to the reinjection of the antigen. In all cases of death, autopsies were done and animals with major hemorrhage from the heart were discarded, as in the histamine experiments.

(*b*) *Results:* Antistine again proved to be the weakest agent in animal experiments. With 10 mg/kg, only 64% of the guinea pigs were protected against anaphylactic shock. In striking contrast to the histamine studies, the other drugs exhibited almost equal effectiveness, inasmuch as a dose of 3 or 4 mg. prevented anaphylactic death in from 90% to 100% of the animals. Certain differences among the drugs appear in the figures, but on a minor scale. (See discussion.)

A number of animals that had survived anaphylactic shock, because of protection by an antihistamine substance, received a second shocking dose 36 hours later. The results were as follows: Of 11 animals that

Antisine or 3277 had protected against one shocking dose, 3 died in anaphylactic shock and 6 had typical shock symptoms, i.e., 82% of the animals could be successfully shocked. Of 8 animals that Chlorothen

TABLE 3

Protection of guinea pigs with antihistamine compounds against active anaphylaxis

ANTIHISTAMINE COMPOUND		NUMBER OF ANIMALS	NUMBER OF DEATHS	SURVIVAL %
Kind	Dose			
	mg/kg			
None (Controls)		12x	12	0
None (Controls)		43	37	14
Antistine	2	4x	4	0
	4	5x	5	0
	10	14	5	64
Benadryl	3	10	0	100
1721 (Searle)	2	10x	3	70
	3	11	4+	64
3277RP	2	9x	3	66
	3	10	2+	80
	4	13	1+	92
Bromothen	3	9	0	100
Chlorothen	3	10	0	100
Histadyl	2	6x	2	66
	3	10	1	90
Pyribenzamine	2	8x	8	0
	3	20	7+	65
	4	9	0	100
Neo-Antigen	2	6	5	16
	3	19 [1]	9	52
	4	10 [2]	2	80
	5	9 [2]	1+	88

x = egg white, as antigen
\+ = delayed death
[1] = in 12 animals, antigen injected intravenously
[2] = antigen injected intravenously.

or Histadyl had protected against two shocking doses, 4 died in anaphylactic shock and 4 had typical shock symptoms. In summation, thirty-six hours after survival from one or two shocking doses, because of protection by one of the antihistamine compounds, 17 of the 19 animals (89%) responded to another shocking dose, 7 animals (36%) with fatal shock.

(*c*) *Discussion:* Most investigators have examined the compounds at their disposal only for their activity against histamine; therefore, very few figures derived through similar technique, and thus suitable for comparison, are available in regard to their anti-anaphylactic effectiveness. Again our results are in sharp disagreement with Halpern and co-workers (14). These authors, using 1 mg/kg Neo-Antergan (4 animals) and 0.25 mg. 3277 (6 animals) reported 100% survival from active anaphylactic shock induced by sheep serum. With 0.1 mg. 3277, they obtained 80% survival in ten animals. Arbesman and co-workers (15), as well as Rose and coworkers (9), tested some compounds against *passive* anaphylaxis and achieved protection with somewhat smaller doses of Pyribenzamine and Neo-Antergan than we found necessary in our studies. Mayer and co-workers (16), who used Pyribenzamine spray preceding the shocking dose, likewise found the protection greater and more consistent in animals passively sensitized than in those actively sensitized. How difficult it is to obtain identical results with identical methods is seen by comparing the figures of Rose and co-workers (9) who had 70% survival following 2 mg/kg Pyribenzamine and 100% survival following 3 mg/kg of this drug, with those of Arbesman and co-workers (15), who (in a small series) had 100% survival following 2 mg/kg Pyribenzamine. Therefore, too much importance should not be attached to differences which might disappear by increasing the number of animals in a given series.

Lehman and Young (17), working with diethylaminoethyl-dihydroanthracen-carboxylate, observed that sensitized guinea pigs which this substance had protected against 50 times as much antigen as had killed 9 of 10 controls did not show any symptoms when they received a subsequent injection of the same dose of antigen two days later. They concluded that the animals had been desensitized by the first shocking dose; therefore, they presumed that the protective effect of their compound was due to its antihistaminic action. Mayer (16) noticed that after protection with Pyribenzamine against anaphylaxis, the animals were still desensitized, and persisted in a state of antianaphylaxis eight days after the first antigen dose. A "silent antibody reaction" seems to have taken place. Such an explanation would be in accordance with observations on the sensitized intestinal strip formerly made by Ackerman and Wasmuth (18) and by Landau and Gay (10), using arginine as

protective agent, and by Rosenthal and Brown (19), using 929F. Ackerman and Wasmuth (18) suggested that the antigen-antibody reaction takes its course unimpeded by arginine, but that the amount of histamine liberated by the antigen-antibody reaction is counteracted. Our own antianaphylaxis studies failed to confirm these in vivo observations, for 36 hours after survival from anaphylactic shock because of protection with various compounds, 17 of the 19 guinea pigs could be shocked. Staub, Halpern, and Vallery-Radot and co-workers (20) reported the same observations. The last named authors repeated the shocking dose after 80 hours and obtained reactions; hence, they concluded that the injection of 3277 had inhibited not only the manifestations of shock but also the reaction of the antigen with the antibody. Staub had obtained fatal shock within one, two, or seven days after the animals had survived the first shocking dose, because of protection with 929F. In view of the fact that Lehman and Young (17) obtained their widely different results by using very large doses of antigen, a quantitative factor might be involved.

CONCLUSIONS

Our studies show that there is a wide difference in potency among these nine so-called antihistamine compounds when tested against one lethal dose of histamine injected into the bloodstream. The scale of effectiveness approximates the activity against the histamine-induced contraction of the intestinal strip of guinea pigs. In striking contrast, all the substances except Antistine proved to be almost equally effective against anaphylaxis. The dose required for protection against anaphylactic death was considerably higher than that required for protection against one lethal dose of histamine; naturally, this difference became the greater, the smaller the protective dose against histamine. The need for higher protective dosage against anaphylaxis than against histamine has been stressed before by two of the present writers (10) and it has been reiterated by Lehman and Young (17).

We believe that our comparative quantitative studies supply the evidence which Loew (21) felt to be lacking to prove this point. Loew (21) stated that this difference in the required dosage would not constitute evidence that factors other than histamine were involved to an important degree in anaphylaxis. On the basis of our observations

with arginine and histidine, we ourselves suggested "that histamine liberated by the antigen-antibody reaction is in more intimate association with the tissue," consequently, more effective than histamine added from the outside.

Why is the potency against histamine so widely different, but the dosage for antianaphylactic effect so very similar? Could it be that these substances, although very effective against histamine, and this in very different degree, also have in higher dosage an antagonistic effect, and this in equal degree, against some other substance involved in anaphylaxis?

There is general agreement that histamine is only one of a number of substances released as the result of an antigen-antibody reaction. We know that heparin is set free, but not much is known concerning other substances which may be liberated.

Dreisbach (22) demonstrated that Pyribenzamine and Benadryl fail to inhibit the Arthus phenomenon in rabbits, an observation confirmed by Fischel (23) for Pyribenzamine. Dreisbach presumed that the central and the peripheral sensory depressions of these agents are responsible for such antiallergic effects as have been observed. The anesthetic effect of antihistamine substances has been (24) the object of much interest in the recent past (24). Code and co-workers found that this anesthetic effect was independent of the inhibitory action of the drugs on skin wheals. Mayer and co-workers (16), using a 2% Procain spray, could not protect guinea pigs against histamine death, but a 2% Pyribenzamine spray had given this protection. More elaborate studies of this aspect of the question are needed.

The evidence in regard to the ability to shock guinea pigs for a second time after they have survived anaphylactic shock because of protection by an antihistamine substance is conflicting. This question is important, because it may shed light on the mechanism of protection. If the compounds interfere with the antigen-antibody reaction, as is suggested by our observations and those of others, the whole concept of the action of these drugs deserves new examination.

SUMMARY

1. The minimal dose that gave guinea pigs 100% protection against one lethal dose of histamine, administered intracardially, was deter-

mined for nine antihistamine compounds, administered subcutaneously. The effective dose varied from 0.02 mg/kg for Neo-Antergan to 1.5 mg/kg for Antistine.

2. Protection against anaphylactic shock required much higher dosage than protection against histamine intoxication. The protective dose of the various compounds was almost equal, with the exception of Antistine.

3. Of 19 animals that had been protected against anaphylactic shock, 17 could be shocked 36 hours later.

REFERENCES

1. Staub, A. M.: Ann. Inst. Pasteur, **63:** 400, 485, 1939.
2. Halpern, B.: J. Allergy, **18:** 263, 1947.
3. Halpern, B.: Arch. Internat. de Pharmacodyn. et de thérap., **68:** 339, 1942.
4. Mayer, R. L.: J. Allergy, **17:** 153, 1946.
5. Winter, C. A.: J. Pharmacol. and Exp. Therap., **90:** 224, 1947.
6. Litchfield, J. T., Jr., Adams, M. R., Goddard, L., Jaeger, M. S. and Alonso, L.: Bull. Johns Hopkins Hosp., **81:** 55, 1947.
7. Meier, R. and Bucher, K.: Schweiz. Med. Wchnschr., **76:** 294, 1946.
8. Lee, H. M., Dinwiddle, W. G. and Chen, K. K.: J. Pharmacol. and Exper. Therap., **90:** 83, 1947.
9. Rose, J. M., Feinberg, A. R., Friedlaender, S. and Feinberg, S. M.: J. Allergy, **18:** 149, 1947.
10. Landau, S. W. and Gay, L. N.: Bull. Johns Hopkins Hosp., **84:** 55, 1944.
11. Feinberg, S. and Bernstein, T. H.: J. Lab. and Clin. Med., **32:** 1376, 1947.
12. Sherrod, T. R., Loew, E. R. and Schloemer, H. F.: J. Pharmacol. and Exper. Therap., **89:** 247, 1947.
13. Lerman, S. and Goldfeder, A.: Compt. Rend. Soc. de Biol., **141:** 446, 1947.
14. Halpern, B., Hamburger, J. and Debray, C.: Bull. et Mem. Soc. Med. Hopitaux de Paris, p. 607, 1946.
15. Arbesman, C. E., Koepf, G. E. and Miller, G. E.: J. Allergy, **17:** 203, 1946.
16. Mayer, R. L., Brousseau, D. and Eisman, P. C.: Proc. Soc. Exp. Biol., **64:** 92, 1947.
17. Lehman, G. and Young, J. W.: J. Pharmacol. and Exper. Therap., **83:** 90, 1945.
18. Ackerman, D. and Wasmuth, W.: Hoppe-Seyler's Ztschr. Physiol. Chemie, **260:** 155, 1939.
19. Rosenthal, S. R. and Brown, M L.: J. Immunol., **38:** 259, 1940.
20. Vallery-Radot, P., Hamburger, J. and Halpern, B.: Presse Méd., **55:** 311, 1947.

21. Loew, E. R.: Physiol. Review, **27:** 542, 1947.
22. Dreisbach, R. H.: J. Allergy, **18:** 397, 1947.
23. Fischel, E. E.: Proc. Soc. Exp. Biol., **66:** 537, 1947.
24. Code, C. F., Keating, J. U. and Leavitt, M. D., Jr.: Conference on "Antihistamine Agents in Allergy" of The New York Academy of Sciences, Section of Biology, Oct. 3 and 4, 1947.

COMPARATIVE STUDY OF ANTIHISTAMINE SUBSTANCES[1]

III. Clinical Observations

LESLIE N. GAY[2], S. WALTER LANDAU, PAUL E. CARLINER, N. S. DAVIDSON, FRANK F. FURSTENBERG, NATHAN B. HERMAN, WILLIAM H. NELSON, JOHN W. PARSONS AND WALTER W. WINKENWERDER

Therapy in allergic conditions has advanced immensely since, four decades ago, Dale laid the cornerstone of the present efforts in "antihistamine therapy." In adrenalin, butanefrine, ephedrine, neosynephrine, etc., we possess powerful drugs for symptomatic treatment. For bronchial asthma, the introduction of aminophyllin, of oxygen-helium inhalations, and recently of massive doses of penicillin (Gay and Marriot) (1) has supplied additional weapons. Diagnosis by careful analysis of the history and by adequate skin tests will allow elimination of the responsible allergen from the environment, or desensitization treatment, or both, with excellent results in many cases.

Nevertheless, the need for further progress is still widely felt. The sympathicomimetic substances mentioned have unpleasant side effects, and in severe cases they are effective only parenterally. Discovery of the provoking agent or mechanism is not possible in every instance, and desensitization is not always feasible or successful. Therefore, after the failure of histaminase and histamine azoproteine, the introduction of compounds that counteracted histamine effects and anaphylactic death in animals was greeted with high hopes.

The first reports (1942) on the initial French preparation, Antergan, were conflicting, but as a whole they were encouraging. When the first American drug, Benadryl, became available, we used it in 101 cases. The results were inconsistent, the side effects very frequent and disturbing. Other compounds were offered to us in rapid succession. In preliminary screening, we eliminated five because of frequent side reactions or lack of clinical effect. Eight preparations were finally

[1] This investigation has been aided by grants from G. D. Searle and Company, Eli Lilly and Company and the Houston Endowment, Incorporated.

[2] From the Protein Clinic of the Department of Medicine, The Johns Hopkins Hospital.

chosen for comparative clinical study, five of these receiving more extensive trial. They were used in dispensary and private patients. As mentioned in the introduction to this entire study, each drug was assigned to at least two members of our group.[3]

The evaluation of new drugs is particularly difficult in allergic conditions because of the psychoneurotic factor in many cases, because of the fluctuating pollen content of the air during the pollen season or variable degrees of exposure to other agents, because of the unpredictable, sometimes self-limited, character of certain conditions, e.g. acute urticaria. Placebos were substituted extensively for the drugs. A considerable number of patients, particularly in the dispensary, had to be eliminated from the study on the basis of their placebo response.

During a twelve months period, ending on December 1, 1947, 686 patients were treated with the drugs. This figure, however, appears only in the tabulation of the side effects. For comparison of the results in the various conditions, we disregarded very small groups and limited the tabulation to drugs given to a sufficient number of patients suffering from the same condition.

Where to draw the line in considering a patient benefited will always be a matter of somewhat arbitrary decision. We registered our cases under the following groups: No Improvement; Improved: 25%; 50%; 75%; 100%. For the final evaluation, we regarded patients who showed 50% or more relief from their symptoms as benefited.

The dosage of the drugs, with two exceptions, was 50 mg. every four to six hours. In preliminary trials it became apparent that this dose was too small for Antistine. Originally, this drug had been used in 100 mg. doses in Switzerland; accordingly, we gave it in this dosage in all cases listed. Hydryllin contains 100 mg. aminophyllina and 25 mg. diphenhydramine base. On the basis of its antihistamine content, 25 mg. would seem to be the effective dose in this combination.

RESULTS

(*a*) *Seasonal and perennial allergic rhinitis* (Table 1) There was no evidence of a significant difference in the results between seasonal, or

[3] For description of the drugs used, see I. Introduction and Dale Experiments. Bull. Johns Hopkins Hospital, *83*: 331, 1948,

pollen, rhinitis and perennial rhinitis, when the latter was definitely of allergic origin. The two types were therefore considered together. Of 428 cases, 60% to 76% were benefited. The best results were obtained with Pyribenzamine, followed by Hydryllin, Antistine, and Neo-Antergan. Relieved were the itching of eye and nose, the watery discharge and the sneezing paroxysms. Nasal obstruction was rarely influenced. Patients with perennial vasomotor rhinitis of the non-allergic type, characterized by negative personal and family history of allergy, by negative skin tests, and by psychoneurotic background were not tabulated because of their small number. They could not be

TABLE 1

Effect of various antihistamine compounds on seasonal and perennial allergic rhinitis

DRUG	NUMBER OF PATIENTS	% BENEFITED
Antistine	43	70
Hydryllin	97	73
1721 (Searle)	23	65
1913 (Searle)	19	68
Histadyl	63	63
Chlorothen	30	60
Pyribenzamine	51	76
Neo-Antergan	102	70
Total	428	68

helped by any antihistamine drug; those who seemed to be temporarily improved had the same result with placebos.

(*b*) *Bronchial Asthma* (Table 2) 96 cases are listed. The number is small, for it soon became obvious that severe cases of asthma did not respond to the drugs, and had to receive adrenalin and aminophyllin for relief. We had, therefore, to restrict the administration of the drugs in asthma in order to avoid needless prolongation of the attacks. Neo-Antergan and Hydryllin appeared to be most effective in these small series. On the basis of closer analysis and extended experience, the figures of Table 2 have to be qualified by the statement that only mild cases of wheezing could be relieved. It was particularly disappointing that ragweed-allergy victims whose nasal symptoms had responded well to one of the drugs during last year's ragweed season

contracted severe asthma in unusually large numbers at the end of the season and failed to obtain any help from the drugs at this time.

(*c*) *Urticaria and angioneurotic edema* (Table 3) In 53 cases, relief varied from 60% to 89%. Antistine appears here on top of the list. The number of cases is too small to warrant conclusions, except the statement that the highest degree of relief can be obtained in this condition. The response was rapid and complete, especially in urticaria caused by penicillin.

TABLE 2

Effect of various antihistamine compounds on bronchial asthma

DRUG	NUMBER OF PATIENTS	% BENEFITED
Antistine	18	33
Hydryllin	26	46
Histadyl	10	40
Pyribenzamine	15	33
Neo-Antergan	27	48
Total	96	40

TABLE 3

Effect of various antihistamine compounds on urticaria and angioneurotic edema

DRUG	NUMBER OF PATIENTS	% BENEFITED
Antistine	19	89
Hydryllin	10	80
Histadyl	14	79
Neo-Antergan	10	60
Total	53	77

(*d*) *Dermatitis and pruritic conditions of various etiology* (Table 4) Of 56 cases, Antistine and Histadyl benefited in 72% and 70%, respectively. Relieved was the itching, but not the skin disease itself. Cessation of scratching naturally resulted in improvement of such skin lesions as were due to superimposed trauma. There was no evidence that the results in atopic and contact dermatitis differ in this respect. Pruritus ani responded excellently in most of the cases, but in a few there was complete failure.

(*e*) *Total results in all conditions treated* (Table 5) If we limit our evaluation to the five drugs with which large groups of patients were treated, we find that an average of 65.4% of all cases treated was helped by the drugs. The incidence of beneficial results ranged from 62% with Histadyl to 69% with Pyribenzamine.

These figures suggest that there is no fundamental difference in the effectiveness of the various drugs. This impression is confirmed when

TABLE 4

Effect of various antihistamine compounds on dermatitis and pruritic conditions of various etiology

DRUG	NUMBER OF PATIENTS	% BENEFITED
Antistine	29	72
Histadyl	27	70
Total	56	71

TABLE 5

Effect of various antihistamine compounds on all conditions treated

(Summary)

DRUG	NUMBER OF PATIENTS	% BENEFITED
Antistine	110	67
Hydryllin	142	65
Histadyl	119	62
Pyribenzamine	71	69
Neo-Antergan	147	64
Total	589	65.4

we consider those patients who have been treated with different drugs in succession.

The change from one drug to another produced results better than, equal to, or worse than those experienced before. There was no rule, in the sense that one drug was always more effective or less effective than another drug. Of two patients with the same condition, one might benefit from drug No. 1 but not from drug No. 2; whereas, the reverse might be true for the second patient. Table 6 gives a particular illustration, indicating the results in hay fever patients when Hydryllin was replaced on 65 occasions by other drugs.

(*f*) *Side effects* (Table 7) The frequency of side effects in the 686 patients treated with the eight different drugs varied from 13% with Antistine to 42% with 1913 (Searle). The frequency of side effects was also high with 1721 (Searle) and Hydryllin. The second lowest incidence of side effects was noted with Histadyl. 119 of the 686 cases

TABLE 6

Effect on hay fever of Hydryllin compared with other compounds in the same patients

OTHER DRUG	BETTER THAN HYDRYLLIN	EQUAL TO HYDRYLLIN	WORSE THAN HYDRYLKIN	TOTAL
Antistine	—	1	1	2
Benadryl	4	8	6	18
1721 (Searle)	3	3	—	6
Histadyl	3	4	5	12
Chlorothen	1	1	—	2
Pyribenzamine	7	7	7	21
Neo-Antergan	2	1	1	4
Total	20	25	20	65

TABLE 7

Side effects of various antihistamine compounds

DRUG	NUMBER OF PATIENTS	% TOTAL SIDE EFFECTS	% SEVERE SIDE EFFECTS
Antistine	110	13	5
Hydryllin	142	36	18
1721 (Searle)	36	36	25
1913 (Searle)	26	42	6
Histadyl	119	19	6
Chlorothen	35	23	0
Pyribenzamine	71	33	25
Neo-Antergan	147	27	3
Total	686		

complained of drowsiness. Other side effects were dizziness, weakness and fatigue, headache, nervousness, tremor, apprehension, tachycardia, anorexia, nausea, abdominal pain, diarrhea, dryness of mouth, blurred vision, dysuria, urinary frequency. Severity of side effects was more frequent with Pyribenzamine, 1721, and Hydryllin than with the other drugs. The individuality of response, mentioned in the discussion of

total results, holds true also for occurrence of side effects. Many a patient who could not tolerate one drug could change to another drug which caused side effects in other patients but not in him.

DISCUSSION

Our results are in basic agreement with those of most other observers. Arbesman and co-workers, (2) e.g., reported improvement in 75% of their patients with allergic rhinitis when treated with Pyribenzamine. In bronchial asthma these authors found 48% improved by this drug, a figure considerably higher than ours. Bernstein and co-workers (3) found that of the patients with nonseasonal and seasonal allergic rhinitis, about 76% were improved by Pyribenzamine and about 65% by Neo-Antergan; in asthma, only 28% were benefited by Pyribenzamine and only 20% by Neo-Antergan. Fuchs and co-workers (4) failed to find any difference in relief from hay fever between a group treated with ragweed extract alone and a group treated with ragweed extract plus Pyribenzamine. However, they employed a fixed dose of 150 mg. daily in all patients, regardless of severity of their symptoms.

The Committee on Therapy of the American Academy of Allergy (5) has released a report based on 1570 cases treated with Hydryllin. This report shows results less favorable in hay fever and vasomotor rhinitis but more favorable in asthma than our study shows. As the figures have been compiled from the observations of 44 investigators, it is doubtful that they are based on identical methods of evaluation.

The same report gives figures of the results with Antistine. Our observations, as well as those of others, disagree with these figures. Only 24% good and 13% fair results were encountered in the Committee's 114 cases of hay fever. Walton (6) found improvement in 73% of his 44 patients with perennial rhinitis. He treated only 4 cases of seasonal allergic rhinitis, all of which improved. 80% of his urticaria patients improved. Britton (7) reported benefit from Antistine in 76% of his allergic patients. Hughes (8) had good results with Antistine in 59% of 32 cases with various allergic conditions.

Feinberg and Bernstein (9) studied the effect of Histadyl. 70% of patients with seasonal hay fever, but only 40% of those with nonseasonal vasomotor (allergic?) rhinitis, were relieved. In urticaria and dermatitis, their results were somewhat less favorable than ours.

The incidence of side effects depends upon the dosage employed. Britton, e.g., noticed side effects with Antistine in 37% of the patients, 13% severe, obviously because he gave 200 mg. of the drug four times a day. With the same drug, Walton had 25% side effects, less than 6% severe; Hughes reported reactions in only 18%, almost all mild. When in our study we correlated the dosage level with the incidence of side reactions, it became apparent that the critical dosage of Antistine (where the reaction curves swing up) was 400 mg. daily.

This critical dosage was 100 mg. for Hydryllin and 200 mg. for Histadyl. Beginning with these doses, the frequency of reactions increased in our cases.

Several cases of dermatitis due to Pyribenzamine have been reported. (Arbesman and co-workers (2), Epstein (10), Harris and Shure (11).) We have not seen such reaction to any of the drugs. Exacerbation of asthma from use of Pyribenzamine has been noticed by Henderson and Rose (12). A similar observation had been made in several instances with Benadryl (Waldbott (13), Levin (14)).

CONCLUSIONS

The results of our study of the so-called antihistamine substances demonstrate that no parallelism exists between their effectiveness against histamine in guinea pigs and their effectiveness against human allergy. The wide differences in potency found in histamine experiments are not found in clinical use. However, there may be a relationship between clinical effect and effect in animal anaphylaxis. Only Antistine required a much higher dose for protection against anaphylactic death than did the other drugs. The same drug is effective in man only when twice as high a dose is used as is necessary with the other compounds. These other compounds gave a very similar degree of protection in animal anaphylaxis and a very similar incidence of benefit in clinical use. Such differences between the drugs as have been seen in both uses are hardly significant.

In selecting a drug for the treatment of an allergic condition, one should consider its side effects as well as its percentage of good results. If it is imperative to avoid drowsiness, as in patients who have to operate engines of any kind, a drug like Histadyl or, when it has become available, Antistine is indicated. Where a sedative effect is

not only non-hazardous but even desirable, a preparation like Pyribenzamine, Hydryllin or Neo-Antergan is preferable, particularly at bedtime. It is fortunate that we are able to choose among several preparations and to change from one to another whenever necessary.

The indications for using the compounds are clear. They cannot replace the diligent search for the etiological factor in allergic conditions and its elimination, if found. While this search is being conducted and in case of failure, these compounds are very helpful adjuvants in allergic rhinitis and urticaria. Their effectiveness in relieving the itching in many conditions makes them particularly valuable; in fact, in this respect they occupy a singular place in our armamentarium. The drugs are not indicated in bronchial asthma, except in the mildest cases. Their administration during an asthmatic attack will unnecessarily prolong the suffering of the patient. All critical observers have emphasized the poor results of these drugs in asthma, a fact that contrasts strangely with their effectiveness against the bronchoconstrictor action of histamine in guinea pigs.

Even in conditions in which we see the highest incidence of beneficial results, complete failures sometimes occur. The nature of such cases deserves closer analysis. The fate of the drugs during their passage through the gastro-intestinal tract should be determined.

Further concentrated efforts are necessary in order better to understand the action of "antihistamine substances," and to eliminate their side effects.

SUMMARY

1. Eight different antihistamine compounds were tested in various allergic conditions.

2. Of 589 cases, 65.4% were benefited. The best results were obtained in urticaria, followed by allergic rhinitis and dermatitis. In bronchial asthma, only the mildest cases were benefited.

3. There were no pronounced differences in the effectiveness of the drugs. The response to the drugs differed from patient to patient. However, a drug which failed or caused severe side effects was frequently replaceable by another drug with good success.

4. Side effects were least frequent with Antistine, although its effective dose was twice that of the other drugs.

5. The differences in potency of the drugs against histamine in guinea pigs were not paralleled by similar differences in clinical results.

REFERENCES

1. Gay, L. N., and Marriott, H.: Tr. & Studies of College of Physicians of Philadelphia, **15:** 3, 1947.
2. Arbesman, C. E., Koepf, G. F., and Lenzner, A. R.: J. Allergy, **17:** 275, 1946.
3. Bernstein, T. B., Rose, N. J., and Feinberg, S. M.: Illinois Med. J., **92:** 91, 1947.
4. Fuchs, A., Schulman, P. M., and Strauss, M. B.: J. Allergy, **18:** 305, 1947.
5. Report of the Committee on Therapy of the American Academy of Allergy, 1947.
6. Walton, C. H. A.: Canad. Med. Assoc. J., **57:** 315, 1947.
7. Britton, C. J. C.: Lancet, **253:** 870, 1947.
8. Hughes, R. F.: Canad. Med. Assoc. J., **57:** 323, 1947.
9. Feinberg, S., and Bernstein, T. S.: J. Lab. and Clin. Med., **32:** 1370, 1947.
10. Epstein, E.: J. A. M. A., **134:** 78, 1947.
11. Harris, M. C., and Shure, N.: J. Allergy, **18:** 408, 1947.
12. Henderson, A., and Rose, B.: Canad. Med. Assoc. J., **57:** 136, 1947.
13. Waldbott, G. L.: J. Allergy, **17:** 142, 1946.
14. Levin, S. J.: J. Allergy, **17:** 145, 1946.